COMPARATIVE EVALUATION OF THE AES-CHEMUNEX LAB BLENDER SMASHER®, SEWARD STOMACHER®, INTERSCIENCE BAGMIXER® AND PULSIFIER® ON VIABLE CELL COUNTS OF FOODS, NOISE LEVEL, ERGONOMICS, AND EASE OF CLEANING

by

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Abstract

Proper microbiological examination of foods involves proper sample preparation in terms of mixing the solid or liquid food with a suitable sterile diluent (usually a 1:10 dilution) in a sterile bag and homogenizing them manually or by means of an instrument. This thesis addresses the effectiveness of Stomacher®, Pulsifier®, Bagmixer®, and Smasher® instruments in terms of: 1) Number of viable cell counts/g of ten food types, 2) Noise level of the four instruments ascertained by a) human and b) decibel meter at five feet (1.52 meters) from each instrument, 3) Ease in cleaning the instruments after use, and 4) Ergonomics.

Following the ISO Method (7218:2007), 25 g each of alfalfa sprouts, spinach, peanuts, ground beef, fish meat, hot dogs, tofu, milk, chicken wing meat, and chicken drum stick meat were placed individually in a sterile sample bag containing 224.5 mL of 0.1% Peptone water plus 0.5 mL of *E. coli* inoculum diluent. Each food was homogenized for 60 seconds in each of the instruments. During each treatment four laboratory workers standing at five feet (1.52 meters) from the instrument assessed the noise level as: very quiet, quiet, nearly quiet, acceptable noise, and loud. Also the noise level was monitored instrumentally by the use of a decibel meter and recorded as Db. Ease of cleaning and ergonomics were determined with the aid of a subjective scale set with the Stomacher® as a reference point.

The results indicate that all four instruments have similar performance in regards to viable cell counts. However, in regards to noise level, the Smasher® and the Bagmixer® are the quietest compared to the Stomacher® and then the Pulsifier®. The Smasher® is also the instrument with the highest ranking in ease of cleaning and ergonomics.

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CHAPTER 1 - Review of Literature

In “Chapter 1: Sample Collection, Shipment and Preparation for Analysis of Compendium of Methods for the Microbiological Examination of Foods,” Gabis et al. (1976) elaborate on the essential considerations to prepare a food sample for microbiological analysis. It is noted that proper microbiological analysis of food samples is heavily dependent in factors such as: collection, transportation, and preparation. The validity of the data that results from the microbiological examination of foods is in direct relationship to the quality of the sample analyzed. In light of these considerations, factors such as temperature of the sample are to be taken with special care. More specifically, for non frozen foods, refrigeration in the range of 0 to 4.4 ºC is recommended. In the case that the food to be analyzed is already frozen, it is best to keep the food in a frozen state. As a general rule, microbiological analysis must take place within a time range of 36 hours after collection of the sample.

The instruments used to collect the samples need to be in sterile condition. In practical terms, this means that laboratory instruments such as: dippers, sampling tubes, syringes, and the like need to be sterilized prior to use. The sample containers used in carrying a microbiological examination protocol needs to be sterile as well. Additionally, the sample containers need to be leak proof, and of appropriate size. Because of their fragile condition, glass containers are not the best choice to serve as sample containers (Gabis et al., 1976).

A range of 25 to 50 grams is observed to be the best range for sample size. The baseline sample size is 10 grams. When dealing with frozen samples, use a sample size of 50 grams. Moreover, the homogenization process can be carried out by a blender or an automated instrument such as the Stomacher®. In case of using a blender, the only special consideration is that a sterile sample container is required for the blending. When using the Stomacher®, however, the sample container used is a sterile polyethylene plastic bag. Upon addition of diluent liquid, the polyethylene plastic bag with the sample in it, is subjected to the homogenization forces of the stomacher for a period of 30 to 60 seconds (Gabis et al., 1976).

Challenges are associated when using sample containers made of polyethylene material. For instance, materials in the sample, such as bone splinters can have sharp edges that perforate the sample container and lead to loss of volume. Upon homogenization, a dilution factor of 1 in

10 , for example 1ml of sample into 9ml of diluent, is recommended. The choice of diluents is wide and dependent on the particular type of food. The most popular diluent suspensions are: Butterfield's phosphate buffer, phosphate buffer with citrate, and 0.1% peptone water. Particular organisms might require less conventional diluents. For example, when analyzing for *Vibrio parahemolyticus* in food samples the use of 3% sodium chloride diluent suspensions is recommended (Gabis et al., 1976).

For those samples that are able to mix in water, shake in either a horizontal or vertical direction for a total of 25 times. This manual shaking procedure ideally takes place through a 1 foot arc in 7 seconds. On the other hand, mechanical devices can be used to mix the choice of diluent and the sample liquid for 15 seconds (Gabis et al., 1976).

In regards to the initial homogenization of the food sample, the essential requirement for an automated homogenizer is that it must "shake the container 25 complete up and down movements of about 1 foot in 10 seconds" (Leininger, 1976).

It is also important to notice that the distribution of microorganisms in a food sample vary in accordance to the physical state of the food. For example, in liquid foods, the natural microflora is distributed with a greater degree of dispersion than in solid foods for example. Moreover, microorganisms can be removed from the surface of fruits and vegetables by means of washing. However, in some foods, the microorganisms are housed in the inner parts of the food matrix. The use of a blender is proposed as a means to break up the food matrix and make the microorganisms available for analysis (Frazier ,1967).

The Association of Official Analytical Chemists of the United States provides specific guidelines as to what are the proper steps for a microbiological examination protocol. In order to provide an example of these guidelines, section 980.31 of the Official Methods of Analysis, elaborates on the materials and methods for the assay of *Bacillus cereus* in foods. The procedure for proper homogenization of foods is described in details "Using aseptic technique, weight 50 g of food sample into sterile blender jar. Add 450 mL phosphate buffered dilution H_2O and homogenize 2 min at high speed (ca 20,000 rpm). Use this 1:10 dilution to prepare serial dilutions from 10^{-2} to 10^{-6} by transferring 10 mL of 1:10 dilution to 90 mL dilution blank, mixing well with vigorous shaking, and continuing until 10^{-6} is reached" (AOAC, 1990).

By means of compressive forces, the bacteria embedded in the matrix of the food sample to be analyzed are removed when using automated instruments such as the Stomacher® (Seward

Laboratory Systems Inc., Bohemia, NY). The disposable sample bag containing the mixed sample and diluent is used to start the dilution schedule. The disposable sample bag is far more practical than the reusable blender cups. This advantage is conferred in favor of the disposable bags, since reusable blender cups need to be sterilized upon every use. It is also notice that further benefits of Stomacher™ relative to blenders are the " low noise level, negligible temperature rise, and the small storage space required for bags" (Sharpe and Jackson, 1972).

Other homogenization methods are: ultrasound treatment, vortex stirring, surface scraping, water spraying, vacuum probe, and even electrophoresis. Depending on the particular circumstances, such methods might be more appropriate than homogenization, however, such methods have failed to gain popularity (Sharpe and Jackson, 1972).

Manual methods for homogenizing food samples prior to a microbiological examination protocol present challenges. In any manual method, inconsistency is observed due to human error. In any manual method, the risk of breaching aseptic technique practices is higher than any of the automated methods. Even with the automated methods, considerations have to be made for the pros and cons involved.

In "Comparison of Homogenizing, Shaking, and Blending, on the Recovery of Microorganisms and Endotoxins from Fresh and Frozen G. Beef as Assessed by Plate Counts and the Limulus Amoebocyte Lysate Test," Jay and Margitic (1979) described a comparative study among three homogenizing techniques. In this study, homogenization by hand, by using a Stomacher®, and by using a Waring blender were evaluated. Manual homogenization was compared against homogenization by the use of a Stomacher® in regards to yield of endotoxins and viable cell counts. The homogenization using a Waring blender was not evaluated for endotoxin recovery. Instead, homogenization with a Waring blender was assayed against the Stomacher® and the manual method in regards to the yield of viable cell counts.

Jay and Margitic (1979) revealed that aerobic plate counts did not exhibit significant variation even with the choice of homogenization method when using a sample of fresh beef. However, in regards to the viable cell counts of coliform bacteria, as ascertained by Violet Red Bile (VRB) agar, the Stomacher® produced a higher recovery yield. Furthermore, in regards to the recovery yield of endotoxins, a significant difference was also observed in favor of the Stomacher®. The recovery of endotoxins was made by means of the Limulus Amoebocyte Lysate (LAL) Test.

Homogenization of food samples by the use of the Waring Blender was arguably the first method to gain popularity. Jay and Margitic (1979) indicated that all glassware, utensils, and diluents must be pyrogen free for endotoxin assay. This state is achieved by heating in a dry-air oven at 185°C for 3h, a process which is deleterious to the blade assembly gaskets of the Waring Blender container. Moreover, it is noted that "the second drawback to the use of blending for LAL is the problem of obtaining a particle-free sample for LAL ..". Therefore this method is disadvantageous for such testing.

The blender can be used for fatty foods during two minutes at low speed (ca. 8,000 rpm). Cooled diluent may be used to counteract the heat imposed on the sample during the blending process. Alternatively, the Stomacher® might be used to homogenize a sample instead of a blender (Messer et al, 1992).

In "Stomaching: a New Concept in Bacteriological Sample Preparation" it was reported that "during mixing [when using blenders] some or all of the mixer surfaces contact the specimen and become contaminated, we looked at ways in which labor needed for resterilizing those surfaces could be avoided" . The use of the Stomacher® constitutes a practical solution to this problem, since it employs disposable sterile bags that can be discarded after use (Sharpe and Jackson, 1972).

The principles behind the Stomacher® instrument is under the patent of "Sharpe and Jackson, British Patent Application number 41395/71". The Stomacher® instrument houses the sample with the diluent in a sterile plastic bag. Paddles are used to apply shearing forces to the bag containing the sample. When the instrument is turned on, the two paddles of the instrument, located side by side, pound the sterile plastic bag against the metallic door. Stomachers® use a capacitor type of electrical motor. For a smaller Stomacher® model, a series of wound motors perform the work. The power from either engine is transferred to the paddles via two eccentrics. An eccentric is a disk fixed on the side of a rotating wheel, and is used to convert circular motion into linear. Also a degree of resilence is achieved in the paddles by using rubber connecting rods (Sharpe and Jackson, 1972).

By means of this mechanism, stalling is avoided when samples are not able to be compressed. Also, smooth metal surfaces for the paddles and the door have proven to yield the best results. Several types of flexible materials such as: polyethylene, polyester, cellulose-

polyethylene-polyvinylidene chloride (PVDC) laminates, and rubber were used as sample containers (Sharpe and Jackson, 1972).

The bacteria are removed from the sample by the means of vigorous shearing forces that sweep the liquid from one side to the other, as well as by the series of small compressions the sample has as it is handled by the paddles. In this manner, bacteria present in capillaries of meat tissue are removed. Additionally, the temperature rise during use is not significant, an approximate measurement is 0.8°C/min. for liquids at 37°C. Other mechanical instruments have an approximate temperature rise of 17°C for a period of 2 min (Sharpe and Jackson, 1972).

A Disposable Sterile Bag for Blenders can be found under United States Patent 5564829. The patent describes a disposable plastic bag to house the samples in the blenders during the homogenization procedure. The design of the bag is a two-ply sheet flexible material joined at opposite sides, and upper and lower ends by heat seals. The described design creates an inner sample receiving chamber. Below the upper seal, there is a defined tear off line across the two sheets that made up the bag. When the bag is utilized, this detachable tear-off line must be removed.

The Stomacher® homogenizes the sample and diluent in a sterile plastic bag, and is a preferred method to conventional manual shaking. Additionally the study also exposes that a greater amount of endotoxins is recovered from a sample when using the Stomacher® rather than when using conventional shaking. Finally, when plating inoculated Gram negative bacteria growing in G. Beef sample, the Stomacher® showed competitive advantage in relation to conventional shaking by hand (Jay and Margitic, 1979).

Relative to the Waring Blender, the Stomacher® is considerably quieter and also provides the benefit of insignificant temperature rise. Because the work is performed consistently outside of human interference, and the instrument does not use any sort of sharp surfaces to blend the sample, Stomacher®-type devices provide a safe and reliable homogenization method (Jay and Margitic, 1979).

Another instrument for automated homogenization is the Pulsifier®, an instrument with an oval metal ring where the sample bag is placed. This ring vibrates applying a high frequency on the sample, in addition to shock waves and intense stirring. The process extracts the microbes from the sample surface into the diluent. The Pulsifier® offers the advantage of producing much

less food debris in the sample bag as compared with the Stomacher®, thus being more conducive for Polymerase Chain Reactions (PCR) studies (Fung et al., 1998).

Another stomacher-type of device is the Smasher®. In addition to the conventional pounding principle of Stomacher®-like devices, the Smasher® offers the advantage of having three levels of adjustable speed and time. Also the doors in the smasher are easily retractable for ease of cleaning. By the same token, the device is equipped with a lower chamber to collect any possible leaking or other materials that could leak in case of a pierced bag. The device is operated by placing a sterile bag with sample in the housing chamber, then selecting speed and time of homogenization in the control panel, and finally shutting the door. Once the door is shut, the Smasher® will automatically start homogenizing.

The originality of the Smasher® lies on the "Smasher effect", by which food samples in the sterile bag are pounded at a maximum strength for a short period at the beginning of the operational time. The effect of this principle is a better blending of the food sample, since it is crushed before the peristaltic effect, commonly observed on Stomacher®-like devices. The smashing chamber is made of a steel door and paddles and the background of the chamber is a plastic. The plastic material used in the Smasher's® chamber is Acrylonitrite Butonadiene Styrene (AES CHEMUNEX, 2006).

Lastly, the fourth homogenizer used was the Interscience Bagmixer®. The Bagmixer® has the same operating principle as the Stomacher™ with the addition of some extra features.

The Bagmixer® is equipped with a transparent window in the door that allows visualizing the food sample as it is being homogenized, as well as adjustable controls for time and intensity of mixing.

Noise level

In this study, one of the main parameters for the comparative evaluation of the Stomacher®, Smasher®, Pulsifier®, and Bagmixer® was the noise level. The noise level was evaluated subjectively on an ordinal scale by laboratory personnel, as well as objectively by a decibel meter located five feet (1.52 meters) away from each homogenizer during operation.

In “Principles of Physics,” physicist Frederick Bueche states that, “sound is usually defined as any compressional disturbance traveling through a material in such a way that it is capable of setting the human eardrum into motion, thereby giving rise to the sensation of hearing”(Bueche, 1988).

Sounds need a traveling medium, such as air. It has been demonstrated that sound cannot be heard when the source of the sound occurs in an area with a vacuum. For instance, a bell cannot be heard if it is contained in a vacuum chamber. Sound waves are capable of being transmitted from the source to almost everything in nature (Bueche, 1988).

Sound waves are made up of compressions and rarefactions. A practical example constitutes the emission of sound from a loudspeaker. Within the loudspeaker, a flexible material denominated as the diaphragm displays forward and reverse movements. When the diaphragm moves forward, a compression in the air is created. As the diaphragm retreats, a volume of decreased air pressure is conceived. The decreased air pressure phenomenon that follows after a compression is called a rarefaction. In nature, a sound wave defined by a profile of compressions and rarefactions dissipates from the source. As observed by experimental measurements, the air pressure variations that are positively associated to sound waves are minimal. More specifically, the book points out that “Even for very loud sounds, the pressure variations are only about 0.01 percent of atmospheric pressure” (Bueche, 1988).

Sound waves traveling from the point of origin do so in all sorts of directions away from the point of origin. For example, when dropping a small rock in a pond it can be observed that liquid waves dissipate outwards from the origin. In this setting, the wave crests are represented in the increasingly bigger circles that travel through the water. As the distance from the source of the sound increases, the curvature of these circles decreases. At a given distance far enough from the source, the curvature of the waves is small enough to resemble a straight line (Bueche, 1988).

As these waves of sound travel across a medium, they also carry energy. The energy carried by waves farther away from the source of emission is less than the energy carried by sound waves close to the source. The amount of energy that a sound wave carries varies in accordance with the size of the wave front. For small wave fronts the energy of the wave is spread evenly over a small surface, for bigger wave fronts the energy that the wave carries is spread evenly over a bigger surface (Bueche, 1988).

Under conditions of 0 ºC, the speed of propagation of sound waves in atmospheric air is 331 meters/seconds. It is also noteworthy that for air in the vicinity of room temperature, the velocity of propagation of the sound waves can be measured by the formula: 331.45 +0.61 t. In this predictive model , t stands for temperature measures in degree Celsius (Bueche, 1988). Therefore, at a room temperature of 37 ºC, the tentative velocity of sound propagation is 354 meters/seconds.

Intensity is a measure of the quantity of energy being transported by the sound wave. A statement is made that "Sound intensity is measured as the amount of energy flowing through a unit area per seconds. The area must be perpendicular to the direction of propagation". The measurements of intensity of a sound are in watts per square meter (Bueche, 1988).

Because the spectrum of sound measurements is considerably wide, it is practical to use a logarithmic scale to express the intensity of sound waves. Such a scale is known as the decibel scale. The values on this scale are obtained by the equation: intensity level in Decibels (dB) = 10 log (I/Io). On the cited equation, I stands for the intensity measured in watts per square meter, and Io is the intensity corresponding to a barely audible sound (Bueche, 1988).

References to the intensities of several classes of noises are given as examples for comparison. For example: pain producing sounds have an intensity of 120 dB, a pounding jackhammer 100 dB, busy street with traffic 70 dB, an ordinary conversation 60 dB, the average whisper 20 dB, the rustle of leaves 10 dB, and a barely audible sound 0 dB (Bueche, 1988).

The perception of sounds is dependent in two factors: the intensity, and the frequency. In humans, the optimal range for frequency of sounds is between 20 Hz to 20,000 Hz. Sound waves with a frequency of about 3000 Hz are best perceived by the human hearing mechanism. For sounds with frequencies not in the optimal value, the intensity must be raised in order for the sound to be audible. A practical example is cited below " a sound wave with a frequency of 1000 Hz can be heard when it has an intensity level of about 5 dB. At a frequency of 100 Hz, however,

the sound level must be about 30 dB if the sound is to be audible. Of course, near the frequency limits of audible sound (20 and 20,000 Hz), the intensity must be very large for the sound to be heard ". Additionally, the threshold of intensity that causes pain is not heavily dependent on the frequency of a sound. Exposure to sound waves in the range of 90dB to 120 dB cause damage in humans, regardless of the frequency (Bueche, 1988).

The guidelines in standard 1910.95 of Occupational Safety and Health Standards, an office of the United States Department of Labor regulate sound level exposure, measured in decibels. Sound levels are not considered a hazard, and do not require the use of protective gear when the sound levels fall in the following standards: 90 decibels for 8 hours, 92 decibels for 6 hours, 95 decibels for 4 hours, 97 decibels for 3 hours, 100 decibels for 2 hours, 102-105 decibels for 1 hour, 110 decibels for 0.5 hours, and 115 for 0.25 hours (OSHA, 2009).

Terrestrial vertebrates have developed structures that "trap, amplify, and process sound waves". In humans, these structures are the: outer, the middle, and the inner ear respectively. The authors went further to explain the function of each of these compartments (Starr et al., 2006).

Sound waves from the air, trapped in the outer ear, and then subsequently processed into the middle and inner compartments with the ultimate result of the generation of action potentials. Such action potentials, which constitute the communication pathway in between neurons, are generated by the hair cells in the inner ear (Starr et al., 2006).

The network of neurons that constitute the auditory nerve, serve the function of transferring such action potentials to the cluster of neurons that constitutes the brain. The authors also maintained that hearing loss is a condition that is brought by the hair cells in the inner ear that have sustained considerable damage due to exposure to sounds of high intensity (Starr et al., 2006).

Effects of Noise Exposure

The emphasis of this study was to evaluate the noise level produced by each of the four instruments when in operation. In the frame of the importance of the noise level produced, the effects of noise exposure on people are described. Several studies have been conducted to evaluate the effects of exposure to high intensity noise effects on workers. As a result of such studies, it has been found that high intensity noises can have detrimental effects not only on the hearing capabilities of workers, but also in other systems of the human body as well.

For instance, in the study "Long-term white noise exposure-induced mtDNA deletion in the auditory system," it is noted that high intensity noises caused deletion of mitochondrial DNA in the ears of rats when such noises were experienced for large intervals of time. The study consisted of exposing a noise with 110 dB intensity on 3 month old rats for an interval of four hours a day for a total of 20 days. The results were monitored by the use of a control group of rats that have not been subjected to the high noise intensity. The deletion of mitochondrial DNA was then evidenced in the ears of the rats that participated in the experiment. The rats that were not in the control group became deaf (Han et al., 2007).

Substantial evidence is provided that links mitochondrial DNA deletion to hearing loss and aging as well .Aging and hearing loss in mammals is thought to be brought by the collective increase of mitochondrial DNA deletion in the cells of the body. The collective deletion of a section made up of 4977 base pairs in the mitochondrial DNA has concrete effects on aging as well as hearing loss (Han et al., 2007).

Furthermore, when the cochlea is deprived of oxygen $mtDNA^{4977}$ deletion occurs. As mammals age, a segment of 4834 base pairs in the mitochondrial DNA has been coupled to hearing loss. The deletion of this segment of 4834 base pairs in the genome of mitochondrial DNA of rats is the equivalent of the deletion of 4977 base pairs in humans. During exposure to high intensity sounds, the amount of oxygen and blood flow to the cochlea decreases. The described oxygen starvation can also be brought by aging. A model for hearing loss based on decreased oxygen supply to the cochlea is described as cochlear hypoxia (Han et al., 2007).

In order to understand in greater detail how cochlear hypoxia takes place, the biochemical pathway for the use of adenosine triphosphate needs to be taken into account.

The role of adenosine triphosphate (ATP) on living systems is of vital relevance. ATP is described as “the intermediate adenosine triphosphate which occurs in all known life forms, is the primary cellular energy currency” (Voet et al., 2002).

ATP is made up of an adenosine base that is attached to three phosphoryl groups. The first phosphoryl group is attached to the adenosine base by means of a phosphoester bond. The remaining two phosphoryl groups are attached to the rest of the molecule by mans of phosphoanhydride bonds (Voet et al., 2002).

ATP is produced in the mitochondria via the Oxidative Phosphorylation pathway. In order to synthesize ATP via this mechanism the mitochondria uses over 90 % of the oxygen supply for the entire cell. As a byproduct of the Oxidative Phosphorylation, a small amount of superoxide is generated . The mitochondria is the only organelle that has the ability to carry out transcription and translation independently from the nucleus. The mitochondria possesses its own genome as well as the necessary enzymes to transcribe DNA into RNA, and translate RNA into Proteins. The DNA found in the mitochondria codes for 13 of the proteins essential in Oxidative Phosphorylation (Han et al., 2007).

Mitochondrial DNA is far more prone to errors in transcription and translation than nuclear DNA. Because of the proximity of the mitochondrial DNA to the electron transport chain in the inner membrane of the mitochondria, the enzymes that synthesize DNA are more prone to commit errors. Furthermore, mitochondrial DNA lacks mechanisms to prevent errors in transcription and translation, such as histones as well as DNA repair enzymes. Relative to the nuclear DNA the rates of incidence of errors in protein translation from mitochondrial genome is 10 times higher than for the translation of nuclear genome (Han et al., 2007).

The deletion of large segments of Mitochondrial DNA is believed to be a risk factor for many human diseases. Specifically the $mtDNA^{4977}$ deletion is a critical factor in aging as well as presbycusis. Han et al. noted “Animal studies have demonstrated an age-associated increase in a 4834 bp mtDNA deletion for rats, analogous to the 4977 bp deletion in humans, with progressive hearing loss.” (Han et al., 2007).

Reduced cochlear blood flow is associated with a progression in age as well as loss of hearing sensibility. As a result of decreased supply of oxygen to the cochlea, reactive oxygen metabolites are produced. Parallel to this phenomenon, free oxygen radicals are synthesized during the process of Oxidative Phosphorylation. Both the free oxygen radicals, as well as, the

reactive oxygen metabolites are believed to cause damage to the cell membrane and mtDNA (Han et al., 2007).

Evidence in favor of hearing loss brought about by starvation of oxygen to the cochlea can be corroborated by animal experiments. Animal subjects who experienced high levels of broadband noise for a continuous period of time had large $mtDNA^{4834}$ segments deleted. The deletion of such sequences of mtDNA are also noted with progressive age. A probable explanation for the incidence of hearing loss due to noise exposure is that neurons along the auditory nerve and the auditory cortex of the brain sustain damage by the overstimulation created by constant exposure to high intensity noises (Han et al., 2007).

Adverse effects of noise exposure can also be amplified by the conditions of the environment where the workers perform their tasks. Because sound propagates through air, the presence of absence of compounds in the air can also affect the propagation of the noise. Consequently, the hearing mechanism of workers in this environment can also be affected.

The article "Hearing Loss in Workers Exposed to Toluene and Noise", appeals that organic solvents have detrimental effects on the hearing capabilities of workers. Organic solvents that are believed to have such effects are compounds such as: toluene, xylene, styrene, n-hexane, trichloroethylene, carbon disulfide, and petroleum among others (Chang et al., 2006).

Solvents such as toluene, can cause harm to the auditory capabilities of workers when present at high concentrations in the environment and with only a little exposure time. The chances of permanent hearing damage are significantly increased by the incidence of noise in an environment with toluene, than in an environment with noise only (Chang et al, 2006).

The analysis "The Association between Noise Exposure and Blood Pressure and Ischemic Heart Disease: A Meta-Analysis," argues on the physiological effects of noise exposure. Noise exposure could be a precursor for high blood pressure and an increased risk of ischemic heart disease. However, a concrete cause and effect relationship can not be specified due to the lack of appropriate epidemiological data (Van Kempen et al, 2002).

Effects of noise exposure can lead to an array of adverse consequences to the overall health. Specifically, effects noted can range from: disturbances in the nervous system, insomnia, concentration, and cognitive performance. Noise exposure can also increase the incidence of annoyance in people (Van Kempen et al., 2002).

Van Kempen et al. (2002), reviewed studies that evaluated occupational and community noise levels. The results of the analysis of the occupational noise studies reveal contradictory information and that more in depth research in the area is needed in order to determine a clear relationship for noise exposure and blood pressure in occupational settings.

Noise exposure leads to stress, and the systems of the body are affected in response to the pressure of the stress. The article suggests that the intensity of the noise is carried from the auditory channels to the reticular arousal system and the hypothalamus. In this manner, neuronal and hormonal pathways can be stimulated (Van Kempen et al., 2002).

The article also mentions that in response to stress, the body secretes hormones such as noradrenaline. These types of hormones are used to elicit precursors for increased blood pressure, heart rate, and the onset of other changes to prepare the body to face the stress. Stress can also produce changes in the habits and decisions of the person affected. For instance, the incidence of alcohol consumption, smoking, and use of pharmaceuticals can increase. The analysis suggests that the incidence of heart disease by noise exposure can occur with higher frequency on those individuals who experience aggravation of a preexisting condition, such as cardiovascular disease by means of stress (Van Kempen et al., 2002).

The findings of Passchier- Vermeer, and Passchier (2000) in the study "Noise Exposure and Public Health," offer evidence that supports the range of previously described adverse health effects in workers. Among the adverse health effects are: hearing impairment, hypertension, annoyance, and sleep disturbance.

Hearing loss is the widening of the hearing range. Additionally, the ISO 1999 section 22 explains that people suffer the effects of hearing loss when they are unable to understand normal conversations with low background noise. It is also considered that hearing loss can be exacerbated with age, interaction with chemicals, head injuries ,and hereditary factors (Passchier- Vermeer and Passchier, 2000).

The psychosocial effects of noise exposure are: annoyance, and even psychiatric hospitalization. Of these two effects mentioned, annoyance is the most prevalent one. Annoyance is defined as resentment, discomfort, and related feelings or behaviors whenever noise levels are high enough to cause a disturbance to the worker. Because more knowledge of the endogenous and exogenous factors that might lead to annoyance is necessary, the prediction of the incidence of annoyance is still not an objective process (Passchier- Vermeer and Passchier, 2000).

Sounds with an intensity greater than 55 dB, leads to annoyance in office workers. However, when dealing with constant sounds, annoyance can happen at exposures less than 55 dB for a period of time of 8 hours. For workers in an industrial environment, annoyance is observed at levels above 85 dB. Such conditions can appear at constant day time doses of 70 dB, and night time doses of 80 dB. Moreover, the number of absences increased at sound levels above 75 dB. The number of industrial accidents seems to be proportional to the increase of sound levels to which the workers are exposed (Passchier- Vermeer and Passchier, 2000).

Changes in sleeping patterns can be brought by noise exposure. Sleep is a process that provides a time of replenishment for the brain and the cardiovascular system. It has been evaluated that a noise of 55dB is capable to wake a person indoors. Noise exposure can also lead to decreased ability to perform tasks at the workplace. Noise exposure leads to: "learned helplessness, increased arousal, alter the choice of task strategy, and decrease attention to the task. Noise may also affect social performance, mask speech and other sound signals, impair communication, and distract attention from relevant social clues" (Passchier- Vermeer and Passchier, 2000).

Exposure to noises with an intensity of 85 dB over a period of 8 hours is the maximum dose exposure that does not require hearing protection. If this dose were to be administered over a long period of time, the workers experience a decrease in the hearing sensibility of 5 to 10 dB. However, some individuals will exhibit more severe hearing conditions because of predisposition to them (Passchier- Vermeer and Passchier, 2000).

Further effects of noise exposure are also noted in the developmental stages of the brain. Chang and Merzenich (2003), in the article "Environmental Noise Retards Auditory Cortical Development," explain that in mammals, the region of the brain known as the auditory cortex can be adversely affected by noise exposure.

The study reveals that young rats were exposed to noise and then notes that normal development in the auditory cortex was delayed as a consequence. The study demonstrates that the auditory cortex can indeed be affected by exposure to noise and even suggest that noise exposure can be a risk factor for abnormal child development. After twelve days, the young rats are able to hear, and the auditory cortical area of the brain develops. It is stated that in a period ranging from two to three weeks, this auditory cortex matures to adult stages. During this period

of development, the auditory cortical area of the brain is highly sensible to the acoustic inputs of the environment (Chang and Merzenich, 2003).

In the experiment performed young rats were exposed to constant white noise of 70 dB of intensity, so as to replace the normal sounds in the environment. The mentioned exposure began at seven days after the rats were born, so as to precede the developmental stage of the auditory cortex. The auditory cortex in the rats was then analyzed by means of an electrophysiological map. The images were taken on a time range of twenty four hours after removing the rats from the noise exposure. In this manner, a sample of three to four rats was taken at sixteen, twenty six, fifty, and ninety day old rats. The article specifies that the control group for this experiment consisted of electrophysiological maps of the auditory cortexes of rats exposed to normal environmental noise and of the same age (Chang and Merzenich, 2003).

The gradual specialization of auditory responsive neurons is a prominent characteristic of an auditory cortex that is in developmental stages. Another prominent characteristic is the "loss of tone-evoked responsiveness over a large, broadly tuned anterior region". The results of the comparison of the group of rats exposed to a constant white noise level with the group of rats that were exposed to environmental noise only revealed a detrimental effect on the auditory cortex. It is important to note, however, that such an effect might not always be observed as the rats grow older. For instance, so long as the constant noise exposure is eliminated before the rats reach and age of twenty one days, the auditory cortex is able to achieve a rather normal development (Chang and Merzenich , 2003).

Noise elicits physiological responses through the autonomous nervous system. The physiological responses of the human body to noise exposure over a short period of time include: increased blood pressure and heart rate (Stansfeld and Matheson, 2003).

In the article "Noise pollution: non-auditory effects on health," evidence is presented suggesting that noise exposure might yield to incidence of high blood pressure. The most convincing data from the article is gathered from a collection of occupational studies. In several studies, workers who were exposed to constant noise levels of about 85 dB, demonstrated high blood pressure relative to a control group of non exposed people. (Stansfeld and Matheson, 2003).

High intensity noises lead to the secretion of high levels of noradrenaline and adrenaline. It is noted that noise triggers a general stress reaction in the human body. Noise annoyance is the

most common effect of noise exposure. Additionally, fear and anger can also be caused by noise. Noise exposure is also seen as a disrupting factor of privacy. The degree of annoyance is related to the level of intervention that noise causes with normal activities (Stansfeld and Matheson, 2003).

Noise exposure leads to annoyance. Furthermore, regular activities, such as conversation and other activities involving speech in some manner are adversely affected by airplane noise. Traffic noise has been shown to be the most detrimental effects for sleep. Loudness is a prime factor in determining how harmful noise exposure is. Loudness is a parameter that encompasses intensity, tonal distribution, and duration of exposure. Interestingly, contradictory evidence exists for a correlation on the duration, and frequency of the sound and the quantity of events with regards to annoyance. High frequency noises lead to an elevated feeling of annoyance (Stansfeld and Matheson, 2003).

Adverse effects of noise exposure can be incremented by means of interactions with other factors that cause stress. For instance, the combined effects of working with a cold and noise exposure amounted to alterations on the normal reaction time. The difference in performance in between healthy and sick individuals was negligible in regards to work performance in a quiet environment. However, when such individuals were exposed to a noise exposure level of 70 dB, the productivity of the individuals infected with a cold was much slower. In “Noise pollution: non-auditory effects on health”, the effects of occupational noise and environmental noise are weighted. In the case of environmental noise exposure, annoyance is thought to be the primary outcome. For the case of occupational noise exposure, a raised blood pressure seems to be the primary outcome (Stansfeld and Matheson, 2003).

In the article “Chronic Noise Exposure and Physiological Response: A Prospective Study of Children Living Under Environmental Stress”, the effects of aircraft noise is evaluated for a period of two years in children of nine to eleven years old. Regular noise exposure to aircraft noise resulted in higher resting blood pressure, and release of epinephrine and norepinephrine. These effects are noticed at noise levels smaller than those required to cause hearing loss. The results were drawn by analyzing a group of schoolchildren before and after the operation of an airport in the nearby areas. For a group of schoolchildren residing in the nearby areas of the Munich International Airport, physiological stress reactions were observed. It is noted that in the

study, this group of children had a higher resting blood pressure than a control group of school children in the areas nearby (Evans et al., 1988).

Noise exposure can lead to elevation in arterial blood pressure. Moreover, the condition of hearing loss might be caused by alterations in the metabolism of Magnesium (Altura et al., 1992).

In the experiment that Altura et al.(1992), performed rats were used as test subjects. In one group, the rats had a normal Magnesium diet for a period of twelve weeks. The plasma Magnesium levels were in the range of 0.96± 0.02 mM. The rats on this group were subjected to noise levels of 85 dB(A) for twelve hours per day for 8 weeks, and subsequently, for a noise level of 95 dB(A) for four weeks. In this group, the rats exhibited a noticeable rise in the systolic and diastolic blood pressure. In addition, the Magnesium levels in the plasma serum exhibited a decrease of 15%. Small reductions in Magnesium content were noted in the aortic and portal vein muscles, whereas Calcium levels rose.

For those animals critically deprived of Magnesium, and not exposed to noise stimulus, a considerable rise in the arterial blood pressure was noticed. The Magnesium levels on the vascular tissue decreased, while the calcium level increased (Altura et al., 1992).

Lastly, for those animals subjected to noise exposure as well as a deprivation of Magnesium for a period of twelve weeks, more radical effects were noticed. For example, the Magnesium levels in the plasma serum, as well as the vascular tissue decreased the most. Coupled with this observation was the incidence of the highest rise in the arterial blood pressure. Additionally, the Calcium levels in found to be in the vascular tissue had the highest increases (Altura et al., 1992).

The results suggest that for low plasma Magnesium levels, constriction of microvessels occurs. Conversely, for high arterial blood pressure, low levels of Magnesium in the plasma serum occur. The velocity of the blood flow through capillary arteries were lowered in correlation with lowered Magnesium levels in the plasma serum. The overall trend in the experiment is that noise exposure, at levels equal to or above 85 dB(A) for extended periods of time, can lead to a clear rise in the arterial blood pressure. In tandem with this phenomenon, it is observed that Magnesium levels in the plasma serum drop, while the calcium concentration in tissues increases. The adverse effect of noise exposure on arterial blood pressure is gradually made worse by dietary deficiencies of Magnesium (Altura et al.,1992).

Pharmaceuticals were used to induce a Magnesium rich or deficient state in the rats and the blood pressure was measured by surgical techniques. The careful design and implementation of the experiment amounts to the validity of a correlation between noise exposure and the rise in arterial blood pressure. In the experiment performed, male Wistar rats of weights between one hundred and one hundred and thirty grams were treated with a drug called Altromin C1035 for a period of twelve weeks. Altromin C1035 is a pharmaceutical used to induce a reduction in the levels of Magnesium in the body. The drug has a Magnesium content of 5 mmol/kg, and a Calcium content of 180 mmol/kg (Altura et al.,1992).

A group of rats under this treatment were given triple distilled water with 4 mmol/kg of Magnesium Chloride per liter. This water intake was aimed at inducing a fair lack of Magnesium in the rats. Another group was fed with triple distilled water without Magnesium Chloride, with the aim to cause a greater Magnesium deficiency in the rats. Another group was used to be the control. Rats in this group received an intake of Altromin C1035, containing 80 mmol/kg of Magnesium, as well as, 180 mmol/kg of Calcium. Additionally the rats in this group were fed with triply distilled water lacking Magnesium Chloride for a period of twelve weeks (Altura et al., 1992).

The rats in the control group were fed in pairs so as to prevent differences in weight. Subgroups of rats were taken from the designated groups and subjected to noise exposure performed in an echoic room. In the initial eight weeks the rats were exposed to noise of 85 dB(A) of intensity from 8 am to 8 pm. The impulse of the noise was activated at random times within the exposure time range. The peak levels of such impulses were of 80, 90, and 100 dB(A). In the last four weeks of the experiment the noise exposure was raised to an intensity of 95 dB(A), for a total exposure time of sixteen hours. High frequency loudspeakers were added so as to increase the spectrum of the noise exposure to intensities of about 80, 90, and 100 dB(A). After the course of eight weeks the condition of noise induced hearing loss was not noted. Consequently, the remaining four weeks of the experiment were carried with a higher intensity of noise exposure with the aim to cause noise induced hearing loss. The rats were housed in rooms with fluorescent lightning from the time range of 8 am to 4 pm (Altura et al., 1992).

Upon completion of the experiment, all rats were anesthetized with pentobarbital sodium, or ketamine hydrochloride. The drugs used to deliver the anesthetic effect were Nembutal and Ketalar respectively. Barton et al. (1992), assayed the effects on blood pressure and

microcirculation by means of surgical techniques. It is explained that the blood pressure measurements were taken by inserting a metal tube, a cannula, into the femoral artery. The cannula was then connected to a device known as the Stathan pressure transducer.

The study reports that after the measurements for the arterial blood pressure were taken, arterial blood samples were taken, and subsequently analyzed. The procedure for analyzing the arterial blood samples was to store the samples into heparinized tubes, then centrifuge at 3000 rpm for a period of ten minutes, and then freezing the samples at temperatures of -70 °C. This protocol was followed so that the Magnesium content could then be assessed by means of atomic adsorption spectrophotometry (Altura et al., 1992).

The aortas and portal veins were taken out, and made free of connective tissue debris. The extracted veins were then frozen at -70 °C. After the freezing the veins were then thawed, and later dry washed at 525 °C, for a period of 24 hours. After this procedures the Magnesium and Calcium contents were assessed via the atomic absorption spectrophotometry technique. The condition of hearing loss or hearing impairment is thought to be caused by the tightening of the veins that supply blood to the cochlea. Another cause of hearing loss can be found in low supply of energy to the cochlear hair cells. Noise exposure can indeed lead to an elevation of the systolic and diastolic blood pressure in test animals. In the study it is shown that low levels of Magnesium in the body can lead to an increase in the arterial blood pressure. It is also shown that hypertension is made worse by Magnesium deprivation (Altura et al., 1992).

Irregularities in the normal metabolism of Magnesium can lead to alterations in the contractile function of vascular smooth tissue, and ultimately in arterial blood pressure. The study illustrates the importance of Magnesium and the biochemical pathways in which it takes place. The importance of Magnesium in the vascular tissue is attributed to the ability of Magnesium cations to regulate the passage of Calcium cations across the cell membrane. Additionally, Magnesium is noted to confer membrane stability in cells. Magnesium receptors in the membranes of the cells that make up the blood vessels play a regulatory role in the entry and exit of Calcium cations. Decreased levels of Magnesium leads to the effect of a high intracellular concentration of Calcium in blood vessels. Magnesium cations are believed to be blockers of the channels in the cell membrane that allow passage of Calcium cations (Altura et al., 1992).

The study elaborates on the effects of high Calcium concentration in cells as a result of low Magnesium levels. The high Calcium concentration leads to a decreased ability to relax in

microvascular smooth muscle. In this manner Calcium cations make the microvascular smooth muscle less responsive to the relaxing agent histamine. The study presents a tentative model as to how elevated noise exposure leads to decreased Magnesium levels in the cells. It is explained that exposure to high intensity noise causes the Magnesium in the cells to be released. A parallel explanation is that exposure to high intensity noises leads to the release of hormones such as catecholamines and adenosine 3',5' cyclic monophosphate. These hormones, in turn, might induce the release of intracellular Magnesium (Altura et al., 1992).

Governmental Regulations on Noise Exposure

Government standards for occupational noise exposure are regulated in close detail under the guidelines of the Occupational Safety and Health Administration. More specifically section 5 (a) (1) of the General Duty Clause grants that employers must provide a work environment without hazards that can cause death or serious adverse health effects. Industry guidelines on noise exposure can be found in chapter 29 of the Code of Federal Regulations, subsection 1910.95 (OSHA, 2009).

Subsection 1910.95 (b) (1), reflects that when workers experience noise exposure beyond the parameters specified as Permissible Noise Exposure, administrative or engineering measures must be taken to protect the workers. Given that such controls are not effective in reducing the noise exposure to a range within the specified Permissible Noise Exposure, hearing protective equipment must be utilized (OSHA, 2009).

Guidelines 1910.95 (b) (2), comments that when noise exposure is experienced at different levels, the joint effect of the exposures is to be considered. In order to quantify such an effect, the following equation is advised: C(1)/ T(1) + C(2) /T(2) = C(n)/ T(n). Where C(n) stands for the time of exposure at a certain noise intensity, and T(n) stands for the time of exposure allowed for that level of sound intensity. In the case that C(n)/ T(n) is greater than 1, then the combined noise exposure is considered to be higher than the Permissible Noise Exposure. Guidelines 1910.95 (c) of Chapter 29 of the Code of Federal Regulations, observes the Hearing Conservation Program. In the subsection 1910.95 (c) (1) it is stated that the employer must establish a hearing conservation program when noise exposure is equal to or greater than an eight hour time weighted average sound intensity of 85 dB, as measured by the A scale (for slow response). The A scale is found in subsection 1910.95 (a) and it displays the equivalent sound intensities for sounds measured using the octave band analysis into A- weighted sounds (OSHA, 2009).

Furthermore, subsection 1910.95 (i) (2) (ii) specifies the use of hearing protectors for workers exposed to an eight hour time weighted average of 85 dB or more. Additionally, in subsection 1910.95 (j) (2), it is established that such hearing protectors must reduce the effective noise exposure to an eight hour time weighted average of 90 decibels (OSHA, 2009).

The Occupational Safety & Health Administration, in section three of the Osha Technical Manual (OTM), exposes that when hazardous noise exposure is at place, workers exhibit change

in normal behavior. Such changes in behavior can be noted in difficulty to communicate with others. For instance, at noise exposures above 80 dB, workers have to speak at high tones, at noise exposures in between 85 and 90 dB, shouting is necessary to communicate, and when noise exposures are above 95 dB, workers must reduce distances so as to be able to communicate (OSHA, 2009).

Additional governmental regulations are examined by "Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998" by the United States Department of Health and Human Services. The article elaborates on the technical definitions of dose and time weighted average in the context of noise exposure. The article defines the dose of noise exposure as the ratio of the intensity of exposure to the amount of permissible noise exposure. In terms of percentages, the dose of the noise exposure is calculated as follows: Dose= (C1/T1+ C2/T2+ …+Cn/Tn) × 100. In the scope of this equation, Cn , stands for the summation of the time of exposure at a given intensity, and Tn, stands for the amount of time for which the given noise intensity becomes hazardous (USDHHS, 1998).

"Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998" also defines the concept of Time Weighted Average (TWA), as " The averaging of different exposure levels during an exposure period. For noise, given an 85- dBA exposure limit and a 3- dB exchange rate, the TWA is calculated according to the following formula: TWA = 10.0 × Log (D/100) + 85, where D= dose" (USDHHS,1998).

The article states that sound exposure in occupational settings must be controlled so as to be below the specified permissible intensities and times. Such parameters were established by using the formula: T (min) = 480 / 2(L-85)/3, in this formula 3 stands for the exchange rate, T for time, in minutes, and L for noise intensity level (USDHHS, 1998).

"Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998" notes permissible noise exposure parameters similar to the ones listed by the Occupational Safety and Health Administration in directive 1910.95. Following is a contrast in between OSHA standards and the ones cited by the Criteria for a Recommended Standard. For an interval of 8 hours, a value of 85 dBA is cited versus the 90 dBA from the OSHA standards. For an interval of 4 hours, a value of 88 dBA is listed, versus the 95 dBA from the OSHA standards. For an interval of 2 hours, a value of 91 dBA is listed, versus the 100 dBA from the OSHA standards. Finally, for an interval of 1hour, a value of 94 dBA is listed, versus the 105 dBA from the OSHA

standards. It can be noted from the comparison that the official OSHA standards for permissible noise exposure are higher than the counterparts listed under “Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998” (USDHHS, 1998).

Recommendations for the layout of a hearing loss prevention program are granted. It is noted that when a Time Weighted Average of 85 dBA for 8 hours is exceeded, a hearing loss prevention program must be in place. As described by the article, a hearing loss prevention program must be composed of several elements. An effective hearing loss prevention program must have initial, as well as, periodic monitoring. This monitoring is to be carried out with calibrated decibel meters with the Slow response selected. In the context of the article, Periodic Monitoring is to be carried out with a frequency of 2 years, and with a frequency of 3 months if there is new instrument or changes in maintenance routines (USDHHS, 1998).

As part of the program employees are to use hearing protectors with the purpose of attenuating the noise exposure level. Medical assistance is to be rendered in order to perform audiometric tests. The audiometric tests can be carried out by physicians, or by an occupational hearing conservationist certified by the Council for Accreditation in Occupational Hearing Conservation. Additionally, warning signs must be used to indicate that there is a noise exposure hazard in a given area. Workers are to be made aware of the tentative consequences of exposure to hazardous noise exposure levels and the use of hearing protectors. A training program is to be set for all the affected by noise intensity levels beyond the permissible limits (USDHHS, 1998).

Finally the last two components of the program are the evaluation, and the recordkeeping components. Evaluation of the program is to be made with the aim that the annual audiometric tests for the workers do not reveal an increase in the hearing threshold of workers. The recordkeeping component refers to the availability of the exposure assessment ,and medical surveillance records, as well as retention of records for at least 30 years (USDHHS, 1998).

An estimate of the number of workers exposed to hazardous levels in the United States is presented. The article declares that “ in 1981, OSHA estimated that 7.9 million U.S. workers in the manufacturing sector were occupationally exposed to daily noise levels at or above 80 dBA [46 Fed. Reg. 4078 (1981a)] ” (USDHHS,1998).

The article declares that in 1981, the Environmental Protection Agency calculated that more than 9 million workers were experiencing noise intensity levels beyond the 85 dBA mark. Some of the estimates are: for agriculture workers, a number of 323000 people affected, for

manufacturing and utility workers, a number of 5.124.000 workers, and for transportation workers, a number of 1.934.000 workers (USDHHS, 1998).

The article describes the decibel meter. A decibel meter is an instrument to measure the intensity of a given sound. The components of a decibel meter are: a microphone, a frequency selective amplifier, and an indicator (USDHHS, 1998).

The article considers that humans are able to hear noises in the vicinity of 4000 Hz better than low frequency noises. The decibel meter has fast and slow switches , that correspond to the exponential averaging. When the switch is set on fast, it means that the measurements will be taken with respect to a 125- milliseconds time constant. When the switch is set on slow, it means that the measurements of the intensity of the noise will be taken with respect to a 1 seconds time constant. In the chapter it is suggested that for the case of sound exposure measurements in occupational settings, the switch is to be set at slow (USDHHS, 1998).

"Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998", observes recommendations for the use of a decibel meter or a dosimeter to quantify noise exposure. The article specifies that for the case of workers who do not move frequently, and experience continuous sounds, a decibel meter is recommended. However, a noise dosimeter is suggested when the workers move frequently and experience noises that are intermittent, or impulsive (USDHHS, 1998).

The article defines a noise dosimeter as a decibel meter with the ability to carry out the Time Weighted Average calculation. The exponential Time Weighting switch option, either fast or slow should not make a significant impact in the readings of the dosimeter so long as a 3 dB exchange rate is selected (USDHHS, 1998).

Different noise intensities are observed for different parts of the body. In light of this information, the American National Standards Institute 1996a, illustrates that the microphone to be used in order to quantify the dose of noise exposure is to be placed in the middle part of the shoulder that is experiencing the highest noise exposure. Moreover, such microphone must be in parallel with respect to the plane of the shoulder (USDHHS, 1998).

A detailed discussion of hearing aids is presented. The article elaborates that a hearing protector is a device with the ultimate purpose of attenuating the intensity of the sound reaching the eardrum. Three types of hearing protectors are the most popular, namely earmuffs, earplugs,

and ear canal caps. In light of the criteria for a hearing protector, devices such as stereo earphones do not offer protection against noise exposure (USDHHS, 1998).

When a time weighted average of 100 dBA is exceeded, earplugs as well as earmuffs must be worn. Even with such combined protection, the attenuation of the noise exposure will amount to only 5 to 10 dB (USDHHS,1998).

In terms of the article, a given hearing protector must be able to reduce the intensity of noise exposure at the ear to levels below 85 dBA. Besides reducing the intensity of the noise exposure, hearing protectors have other aspects to be considered. The article mentions that in selecting a hearing protectors, a variety of aspects are to be considered. These aspects range from " the workers who will be wearing them, the need for compatibility with other safety equipment, and workplace conditions such as temperature, humidity, and atmospheric pressure" (USDHHS, 1998).

The article cites some reasons for which the workers are not very prone to use hearing protectors. Reasons for neglecting hearing protectors are: discomfort, difficulty understanding speech, and even the belief that no matter what the workers do, Noise Induced Hearing Loss is imminent. However, with proper education programs the cited reasons can be resolute (USDHHS, 1998).

"Criteria for a Recommended Standard, Occupational Noise Exposure, Revised Criteria 1998" argues that the use of such hearing protectors must be constant in order to be effective. For instance " a hearing protector that could optimally provide 30 dB of attenuation for an 8- hr exposure would effectively provide only 15 dB it the worker removed the device for a cumulative 30 min during an 8-hr day." (USDHHS, 1998).

Chapter 40 of the Code of Federal Regulations section 211, requires that the Noise Reduction Rating be reported for each hearing protector. The article describes that the purpose of the Noise Reduction Rating is to measure the effective noise exposure even when using a determined hearing protector. The most successful devices in regards to effective Noise Reduction Rating are earmuffs and earplugs. Furthermore, earmuffs have a higher ability, with respect to Noise Reduction Rating, to reduce noise exposure relative to foam earplugs (USDHHS, 1998).

In order to estimate the effective reduction in noise exposure, the National Institute for Occupational Safety and Health, proposes two methods. In the first method proposed the use of

“subject fit data based on ANSI S12.6-1997 [ANSI 1997]” is noted. The article also expresses that “If subject fit data are Not tested in 1st Rep., NIOSH recommends derating hearing protectors by a factor that corresponds to the available real-world data”. (USDHHS, 1998).

Under the guidelines of the seconds method to estimate the effective reduction in noise exposure, the National Institute for Occupational Safety and Health provides a factor to be subtracted from a given type of hearing protector. The values of effective reduction in noise exposure are to be obtained by the following guidelines: for earmuffs, subtract 25% from the Noise Reduction Rating level reported on the label, for formable earplugs, subtract 50% of the labeled Noise Reduction Rating, and finally for all other earplug devices, subtract 70% from the labeled Noise Reduction Rating. The article provides an example for when the noise exposure is measured in dBA. For this case, the equation that measures the Effective Noise Level (ENL) is: ENL = dBA – (diminished Noise Reduction Rating- 7) (USDHHS, 1998).

As a final note on the topic, the article emphasizes that the ultimate protection from detrimental noise exposure is the physical separation in between the worker and the emission. For the cases in which this is not feasible, the most appropriate hearing protector is the one that the workers will wear constantly. This is so because the effectiveness of a hearing protector is highly dependent on the amount of usage. The article cites a series of characteristics that will make the workers more prone to the use of such devices. The list of characteristics is a follows: “convenience and availability, belief that the device can be worn correctly, belief that the device will prevent hearing loss, belief that the device will not impair a worker’s ability to hear important sounds, comfort, adequate noise reduction, ease of fit, compatibility with other personal protective equipment” (USDHHS, 1998).

Ergonomics

When using automated homogenization methods, it is advisable to use a instrument that offers ease of use for the operators. Ergonomics is an important parameter of this study because it measures how comfortably and easily each automated device can be used. In this study, the level of ergonomics for each instrument was subjectively evaluated by means of an ordinal scale with the following values: 1. High Ergonomics, 2. Acceptable Ergonomics, and 3. Low Ergonomics.

The article "Ergonomics, quality and continuous improvement- conceptual and empirical relationships in an industrial context published in Ergonomics," states that "the Nordic Ergonomics Society defines ergonomics as the Interdisciplinary field of science and application considering integrated knowledge of human requirements and needs in the interaction human-technology- environment in the design of technical components and work systems" (Eklund, 1997).

In the article, Eklund describes that ergonomics and quality are complementary characteristics that are desirable in competitive markets. Moreover, it is observed that safety is an important part of ergonomics. It is also interesting that the author points that the 'Human error' is directly proportional to the increase in safety and quality issues (Eklund, 1997).

An undesirable work environment can ultimately lead to a considerable incidence of quality deficiencies in the product flow. In the study it is portrayed, how undesirable working conditions can lead primarily to effects such as: discomfort, impaired perception, and even mental disorders. Furthermore, discomfort, in turn, leads to: distraction, and compensatory activities. Finally, distraction, compensatory activities, impaired perception, and mental disorders result in the incidence of human error. Human error is then the precursor of quality issues (Eklund, 1997).

In the process flow of conducting a quantitative or qualitative analysis on a microbiology laboratory, a variety of tasks are encountered. Among this variety of tasks, are monotonous procedures such as diluting by a constant factor, and plating a constant volume in a series of Petri dishes.

It is noted that, "Repetitive and monotonous jobs [such as the ones in a laboratory] give rise to symptoms of boredom or fatigue, followed by a decrease in performance in terms of

longer reaction time and an increased error rate progressively during the work periods". In the frame of intertwining ergonomics with quality, J. Eklund, contends that positive outcomes are: " improved product usability, improved user performance, differences among users accommodated, safer product, improved user comfort, enhanced user satisfaction" (Eklund, 1997).

The International Ergonomics Association (IEA), defines in its website that ergonomics is the discipline of scientific study that aims to maximize the health of the workers, as well as, the system performance. Additionally the IEA describes three main streams of ergonomics. The first branch of ergonomics is Physical Ergonomics, which is mainly focused on how the anatomy of human beings interacts with the physical elements of work. For instance, this branch of ergonomics is concerned with topics such as: appropriate working postures, the configuration of the workplace, and the measures related to safety in the workplace to cite a few (IEA, 2008).

The seconds branch is Cognitive Ergonomics, which is focused on the cognitive processes that workers use to realize tasks. Examples of applications of this branch of ergonomics include: decision- making, work stress, and training for tasks. Third, is Organizational Ergonomics, which is primarily concerned with the management hierarchy within an organization. Examples of applications of this branch or ergonomics include: teamwork and cooperative work (IEA, 2008).

For the purposes of the current study, ergonomics is concerned not only with the design of the automated instruments, but also with the use of hearing protectors when necessary. Hearing protectors constitute a practical application to promote the well being and the productivity of workers when high intensity noises are present in the work environment.

For instance, in the Ergonomics hand out provided by the Communications Electronics Command (CECOM) Directorate of Safety Risk Management by the U. S. Army, the use of hearing protectors such as earplugs and noise muffs are specified as applications of measures to apply ergonomics in the workplace when experiencing high intensity noises (CECOM, 2008).

Workers are not fully aware of the consequences of constant use of hearing protectors. It is also important to notice that comfort plays an important role on the use of hearing protectors (Arezes and Miguel, 2002).

Attenuation of noise intensity by means of hearing protectors is in direct proportion to the time of use. For instance, if a worker wears the hearing protection device for only 90% of an 8

hour time period, detrimental effects are noted. In this case, the period of unprotected noise exposure amounts to 48 minutes. The efficiency of the hearing protector device, in terms of protection from noise intensity, decreases from 30 dB, to less than 10dB (Arezes and Miguel, 2002).

Food Samples

In order to evaluate the viable cell count among the Stomacher®, Smasher®, Pulsifier®, and Bagmixer®, 10 different food samples were used. Since the 10 different food samples were all commercial food samples, which may or may not have large enough bacterial numbers for analysis, inoculation of known culture was necessary to obtain a countable range of colonies on the Petrifirms®.

Because Aerobic Plate Count and *E. coli* Coliform Petrifilms were used, the inoculum organism was *Escherichia coli* American Type Culture Collection (ATCC) # 51.813. This organism was grown for a period of 24 hours prior to each repetition of the experiment on Triptic Soy Broth tubes (Difco, Detroit). The cultures were hydrated and incubated into the broth since they came in the form of lypolized pellets.

The Centers for Disease Control and Prevention (CDC) of the U.S. in the Division of Foodborne, Bacterial and Mycotic Diseases, makes reference to *E. coli* as a "large and diverse group of bacteria". The CDC also declares that while the majority of the strains of this bacteria are not harmful to man, some strains are capable of causing serious damage to the well-being of humans. Specifically, upon infection, diarrhea, pneumonia, as well as urinary tract and respiratory complications ensue. Moreover, *E.coli* can be used as an indicator for water contamination (CDC, 2008).

A medical condition, known as the Hemolytic Uremic Syndrome (HUS), is among the most severe complications caused by *E.coli* infections. Persons suffering from HUS, experience a decreased frequency of urination, feeling of tiredness, and discoloration of the cheeks. Such are all symptoms of kidney failure brought by *E.coli* infection. Immediate medical attention is required, and upon treatment most patients recover from the illness (CDC, 2008).

The U.S. Food and Drug Administration, in the Bacteriological Analytical Manual Online, Chapter 4, "Enumeration of *Escherichia coli* and the Coliform Bacteria," specifies that "*E. coli*, originally known as *Bacterium coli* commune, was identified in 1885 by the German pediatrician, Theodor Escherich". The FDA notes that the bacteria's natural habitat include the gastro-intestinal tract of humans. It is also revealed that *E.coli* is under the *Enterobacteriaceae* family (FDA, 2002).

According to the FDA, in the Bacteriological Analytical Manual Online, Chapter 4, "Enumeration of *Escherichia coli* and the Coliform Bacteria," *E. coli* is found at high concentration in human feces and was therefore proposed as an indicator of fecal contamination. Furthermore, another characteristic of this organism is its ability to utilize the sugar lactose as a substrate for energy (FDA, 2002).

The article "Complete Genome Sequence of Enterohemorrhagic *Escherichia coli* O157: H7 and Genomic Comparison with a Laboratory Strain K-12," performs a comparison among these two strains of *E. coli*. The results reveal segments of the genome that are specific to O157: H7 or to K-12 strain, as well as a genome segment that is common for the two. The common segment has a length of 4.1 Mega bases. The article inquires that these two different strains came out to be as a result of a change in the genome sequence of the common region via the intermediate action of bacteriophages (Hayashi et al., 2001).

Lastly, in "Molecular archeology of the Escherichia coli genome," by Lawrence and Ochman (1998), it is exposed that another pathogenic Gram negative bacteria, known as *Salmonella*, is the precursor organism for *E. coli*. The article concedes that the appearance of *E. coli* from the mentioned predecessor took place about 100 million years ago. Different strains of *E.coli* appeared by means of a mechanism known as "Horizontal Gene Transfer".

In order to quantify the total bacterial load in the food samples homogenized by each instrument, Viable Cell Counts were taken. The Viable Cell Count (VCC) procedure was performed to quantify the approximate viable number of bacteria that were present in one gram of a given food sample.

For over a hundred years, Viable Cell Counts have been used to quantify the number of bacteria in a food sample. In an agar medium, bacteria can grow and form colonies that are visible to the naked eye. It is estimated that a visible colony of microorganism in an agar plate has a population of nine billion microorganisms. The results of a Viable Cell Count are to be interpreted in reference to a scale (Fung, 2008).

Because the number of microorganism evaluated by the VCC procedure is very elevated, it is more convenient to express the magnitudes in a logarithmic scale. When the VCC counts range from 0 to 2 logs per gram, then there is no relevant concern for microbial contamination. When the VCC counts range from 3 to 4 logs per gram, then there is ground for a moderate concern related to microbial contamination. When the VCC range from 5 to 6 logs, then a serious

concern for microbial contamination is noted. At values higher than 6 logs per gram of sample, bacterial growth is elevated enough to cause the food to lose its palatability and physical appearance. For example, at 7 logs per gram, the food begins to spoil. At 8 logs per gram, bad odors develop. At 9 logs per gram, slime is present in the food , and finally at 10 logs per gram of sample the food product must be discarded immediately (Fung, 2008).

The exact procedure used to quantify the viable cell count per gram of sample was carried out by the use of a formula. Colony Forming Units (CFUs) were obtained from the examination of Petrifilms upon incubation. The CFUs that were in the countable range of 25 to 250 for APC media, and 15 to 150 for the ECC media were then added and divided by two to obtain the average. The average CFU number was multiplied by a correction factor of 10 to account for the initial dilution of the sample along with the diluent. This number was then multiplied again by the dilution in which the countable range of CFUs were found. The resulting number was then divided by a factor of 25 because weight of the sample was 25 grams.

By means of this calculation, an estimate number of bacteria present in one gram of the sample was evaluated. Because the numbers at which bacterial populations grow is considerably high, it is of practical use to take the Logarithm. The results of the Viable Cell Count procedure were expressed in terms of Logs per gram of sample.

In light of all the parameters used to define this experiment, the goal was to perform a comparative evaluation of all four instruments. The principal aim of the study was to evaluate the comparative similarities and/or differences among the Blender Smasher®, the Interscience Bagmixer® ,the Pulsifier® , and the Stomacher® for Viable Cell Counts of the food under investigation.

CHAPTER 2 - Preliminary Study

Introduction

Preliminary work was performed to determine recovery of natural microflora. The performance of the four instruments was evaluated in regards to: Viable Cell Counts, Noise levels, and Ease of Cleaning. The Viable Cell Count (VCC) was ascertained by the use of Aerobic Plate Count (APC), and *E coli* Coliforms Count (ECC) Petrifilms™ (3M, St. Paul, MN).

The Noise levels were evaluated objectively by using a decibel meter (Music Flash Sound Level Meter. M. 33-1028) located at five feet (1.52 meters) away from the source. Ease of cleaning of each instrument was evaluated by subjective means, taking the Stomacher® as a reference point.

Materials and Methods

A. Viable Cell Counts

1. A sample of 25 g of food was placed with 225 mL of 0.1% Bacto™ Peptone (Becton, Dickinson, and Co., Sparks, MD) water, of each type of food.

2. All samples were treated for 60 seconds at medium speeds of each instrument for each sample.

3. Standard plate counts, as well as coliform counts of each sample were monitored in duplicate by using Aerobic Plate Count (APC) and *E. coli* Coliform Count (ECC) Petrifilm™ media (3M, St. Paul, MN).

In order to obtain a countable range of Colony Forming Units (CFU), a predetermined dilution schedule was used (Table 1). For convenience, abbreviations were used to denominate the food samples used in the experiment (Table 2).

B. Noise Level

Objective evaluations of Noise levels were performed by a Decibel meter (Music Flash Sound Level Meter. M. 33-1028) placed at five feet away (1.52 m). Governmental regulations on noise exposure levels were used as reference for exposure characterizations (Table 3).

C. Ease of Cleaning

Ability to clean level of each instrument was evaluated by four laboratory personnel upon homogenization of all food samples. The ranking used to evaluate Ease of Cleaning was:

1. Very easy, complete and quick cleaning
2. Acceptable cleaning
3. Difficult

D. Statistical Analysis

The statistical analysis of the data sets for the Viable Cell Counts, Noise Levels, Ease of Cleaning, and Ergonomics was performed with the Statistical Analytical Software (The SAS Institute, Cary, NC).

Table 1 Dilution Schedule for the Food samples used

Type of Food	Dilutions to be Plated	Type of Petrifilm™ media (1mL plated)
Ground beef	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Hot dog	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Spinach leaves	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Alfalfa sprouts	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Chicken sticks	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Chicken wings	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Fish meat	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Tofu	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)
Peanuts	10^{-2},10^{-3},10^{-4} 10^{-1},10^{-2},10^{-3}	Aerobic Plate Count (APC) *E coli* Coliform Count (ECC)

Table 2 Abbreviations used for the food samples studied

Food	Abbreviation
Ground Beef	G. Beef
Hot Dogs	H. Dogs
Spinach Leaves	S. Leaves
Alfalfa Sprouts	A. Sprouts
Chicken Drum Sticks	C. Sticks
Chicken Wings	C. Wings
Fish Meat	F. Meat
Tofu	Tofu
Peanuts	Peanuts

Table 3 Governmental Guidelines on Noise Exposure levels (OSHA, 2009).

Duration per day, hours	Sound level Dba slow response
8	90
6	92
4	95
3	97
2	100
1.5	102
1	105
.5	110
.25 or less	115

Results and Discussion

In regards to Viable Cell Counts, the data set corresponding to the preliminary trial of the experiment was analyzed using the GLM command of the SAS (Cary, NC) program. The preliminary study was done without inoculation of an indicator organism since it was thought that the natural microflora of the food samples under consideration would yield Colony Forming Units (CFUs) of bacteria in the countable range for the types of media employed.

Nevertheless, inoculation was later implemented as described in the Materials and Methods chapter, since it provided the advantage of yielding more CFUs in the countable range of the type of media used.

The data set for the Viable Cell Counts of the samples for each of the four Instruments displays the same tendencies than the subsequent inoculated replications of the experiment, with the difference of lower magnitudes in the Logs of bacteria recovered (Table 4). Milk was not analyzed on this initial trial of the experiment.

The Averages of the APC counts as well as the ECC counts also depict the same relationship than their counterparts for the subsequent inoculated replications of the experiment (Figure 1, and Figure 2).

The SAS output using the procedure GLM yielded a mean Logarithmic count of 2.11. This value denotes the relatively low levels of bacterial populations naturally present in the food samples. Additionally, the p-values for the Instrument, and the Interaction between the Instrument and Media variables were: 0.82, and 0.99 respectively. These high p-values provide strong evidence to support the claim that neither the type of Instrument employed, nor any interaction between the type of Instrument used and a particular type of Media had any significant difference in the mean logarithmic count of the foods. The data set in consideration supports the same claim than the data set for the inoculated studies: no particular instruments yielded a significantly higher or lower mean logarithmic count. The data provides strong evidence that no particular instrument is better suited for a given type of media, and that no particular instrument is better suited to evaluate Viable Cell Counts.

According to the analysis, the only significant difference is noted in the analysis of the Media variable, with a resulting p-value of <0.0001. This low p-value denotes that ,on average, the mean value of the Media variable is significantly different for the instruments under consideration. This difference can be attributed to the types of media used, namely, APC, and ECC Petrifilms™.

The statistical analysis shows that the mean logarithmic count for the APC media was 3.13, and the mean logarithmic count for the ECC media was 1.24.

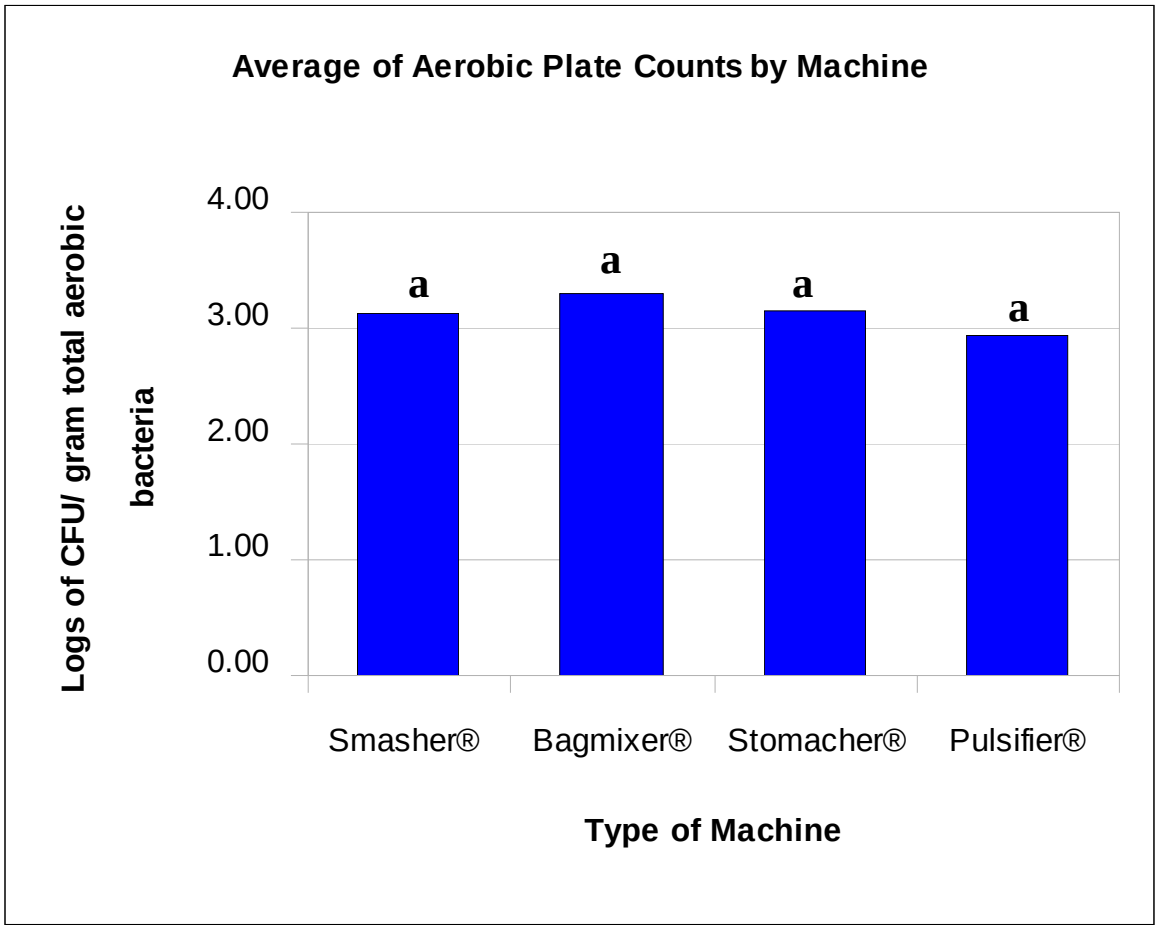
Table 4 Viable Cell Counts for the Preliminary study

Instrument	Food	Media	Logs
Stomacher	G. Beef	APC	**4.76**
Stomacher	H. Dogs	APC	**2.30**
Stomacher	S.Leaves	APC	**4.97**
Stomacher	A. Sprouts	APC	**TNTC**
Stomacher	C. Sticks	APC	**2.30**
Stomacher	C. Wings	APC	**2.30**
Stomacher	F. Meat	APC	**2.15**
Stomacher	Tofu	APC	**TNTC**
Stomacher	Peanuts	APC	**3.30**
Smasher	G. Beef	APC	**4.45**
Smasher	H. Dogs	APC	**1.30**
Smasher	S.Leaves	APC	**4.43**
Smasher	A. Sprouts	APC	**TNTC**
Smasher	C. Sticks	APC	**2.30**
Smasher	C. Wings	APC	**2.30**
Smasher	F. Meat	APC	**2.53**
Smasher	Tofu	APC	**6.43**
Smasher	Peanuts	APC	**1.30**
Pulsifier	G. Beef	APC	**4.51**
Pulsifier	H. Dogs	APC	**1.30**
Pulsifier	S.Leaves	APC	**4.70**
Pulsifier	A. Sprouts	APC	**TNTC**
Pulsifier	C. Sticks	APC	**2.30**
Pulsifier	C. Wings	APC	**2.30**
Pulsifier	F. Meat	APC	**1.30**
Pulsifier	Tofu	APC	**3.82**
Pulsifier	Peanuts	APC	**3.30**
Bagmixer	G. Beef	APC	**4.48**
Bagmixer	H. Dogs	APC	**1.30**
Bagmixer	S.Leaves	APC	**5.49**
Bagmixer	A. Sprouts	APC	**TNTC**
Bagmixer	C. Sticks	APC	**3.30**
Bagmixer	C. Wings	APC	**3.78**
Bagmixer	F. Meat	APC	**2.45**
Bagmixer	Tofu	APC	**4.32**
Bagmixer	Peanuts	APC	**1.30**
Stomacher	G. Beef	ECC	**2.48**
Stomacher	H. Dogs	ECC	**0.30**
Stomacher	S.Leaves	ECC	**2.08**
Stomacher	A. Sprouts	ECC	**2.00**
Stomacher	C. Sticks	ECC	**0.90**
Stomacher	C. Wings	ECC	**2.16**
Stomacher	F. Meat	ECC	**1.38**
Stomacher	Tofu	ECC	**0.30**
Stomacher	Peanuts	ECC	**0.30**

Smasher	G. Beef	ECC	**2.38**
Smasher	H. Dogs	ECC	**0.30**
Smasher	S.Leaves	ECC	**1.30**
Smasher	A. Sprouts	ECC	**0.90**
Smasher	C. Sticks	ECC	**0.30**
Smasher	C. Wings	ECC	**2.00**
Smasher	F. Meat	ECC	**1.56**
Smasher	Tofu	ECC	**0.30**
Smasher	Peanuts	ECC	**0.30**
Pulsifier	G. Beef	ECC	**2.15**
Pulsifier	H. Dogs	ECC	**0.30**
Pulsifier	S.Leaves	ECC	**2.60**
Pulsifier	A. Sprouts	ECC	**2.15**
Pulsifier	C. Sticks	ECC	**0.30**
Pulsifier	C. Wings	ECC	**0.30**
Pulsifier	F. Meat	ECC	**0.30**
Pulsifier	Tofu	ECC	**0.90**
Pulsifier	Peanuts	ECC	**0.90**
Bagmixer	G. Beef	ECC	**1.60**
Bagmixer	H. Dogs	ECC	**0.30**
Bagmixer	S.Leaves	ECC	**5.02**
Bagmixer	A. Sprouts	ECC	**1.48**
Bagmixer	C. Sticks	ECC	**2.30**
Bagmixer	C. Wings	ECC	**0.78**
Bagmixer	F. Meat	ECC	**1.58**
Bagmixer	Tofu	ECC	**0.30**
Bagmixer	Peanuts	ECC	**0.30**

(Table 4, continued)

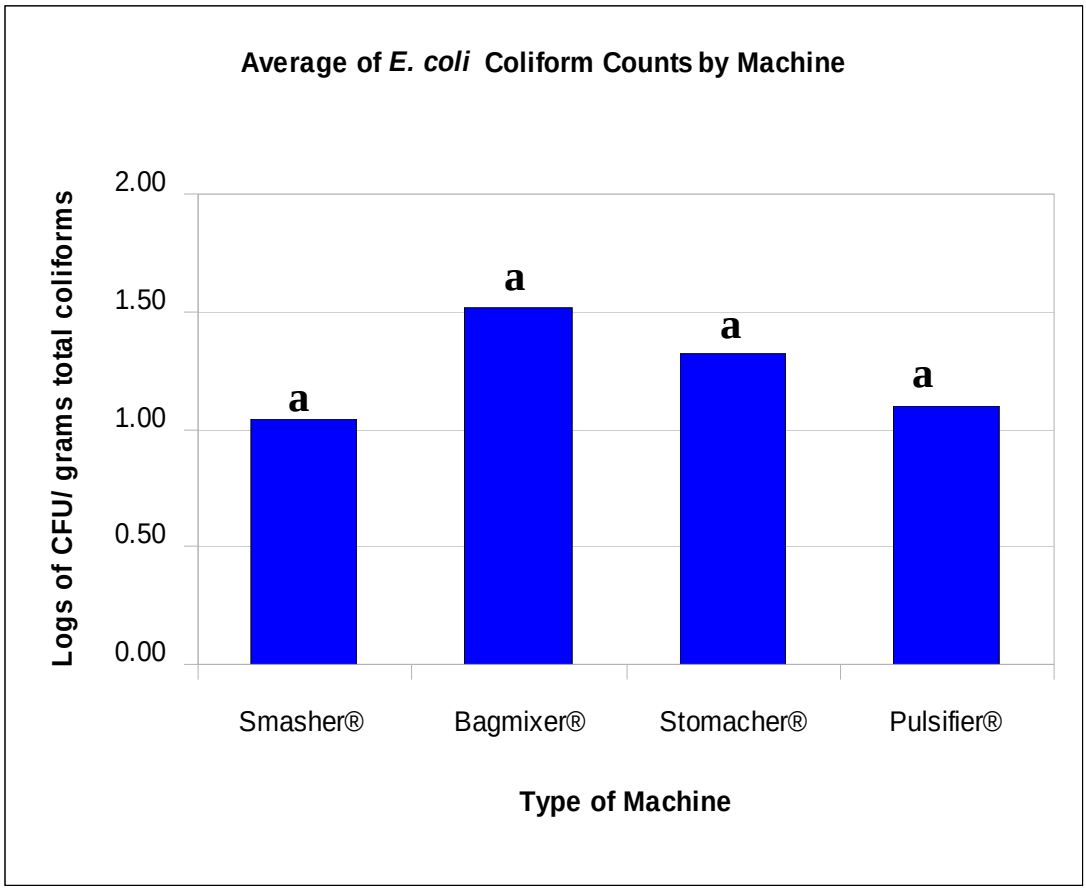
Figure 1 Average of Aerobic Plate Counts, measured as Logs of CFU/g total aerobic bacteria of sample of each instrument for: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Tofu, and Peanuts. N= 9 observations per machine.



[a]Data series in Bars with same letter are not statistically different (P>0.05)

Standard error=0.23

Figure 2 Average of *E. coli*/Coliform Plate Counts, measured as Logs of CFU/g total coliform bacteria of sample of each instrument for: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Tofu, and Peanuts. N= 9 observations per machine



[a]Data series in Bars with same letter are not statistically different (P>0.05)

Standard error=0.21

Decibel emissions for each machine were recorded for each instrument while in operation (Table 5). The decibel emissions recorded were taken with the slow setting, since all government regulations on noise exposure levels correspond to this setting.

In regards to Noise Levels measured by the decibel meter (Music Flash Sound Level Meter. M. 33-1028), a statistically significant difference in decibel emissions was noted (Figure 3). The hypothesis test by SAS revealed a mean decibel emission level of 68.83. The p-value for the instrument variable was <0.0001. These results are consistent with a strong evidence to explain that on average the noise level amongst the instruments is significantly different. The mean value for the decibel emitted by each instrument while in operation was as follows: Smasher ® 63.11, Bagmixer® 66.44, Stomacher® 68.66, and Pulsifier® 77.11.

Ease of cleaning was evaluated by a scale ranging from 1 to 3, with 1 for easy to clean, and 3 for difficult to clean (Table 6).

With respect to the ease of cleaning of each instrument after use, the evaluations ranked the Smasher®, and the Pulsifier® as equally easier to clean than the Bagmixer®. The Stomacher® was the hardest to clean (Figure 4).

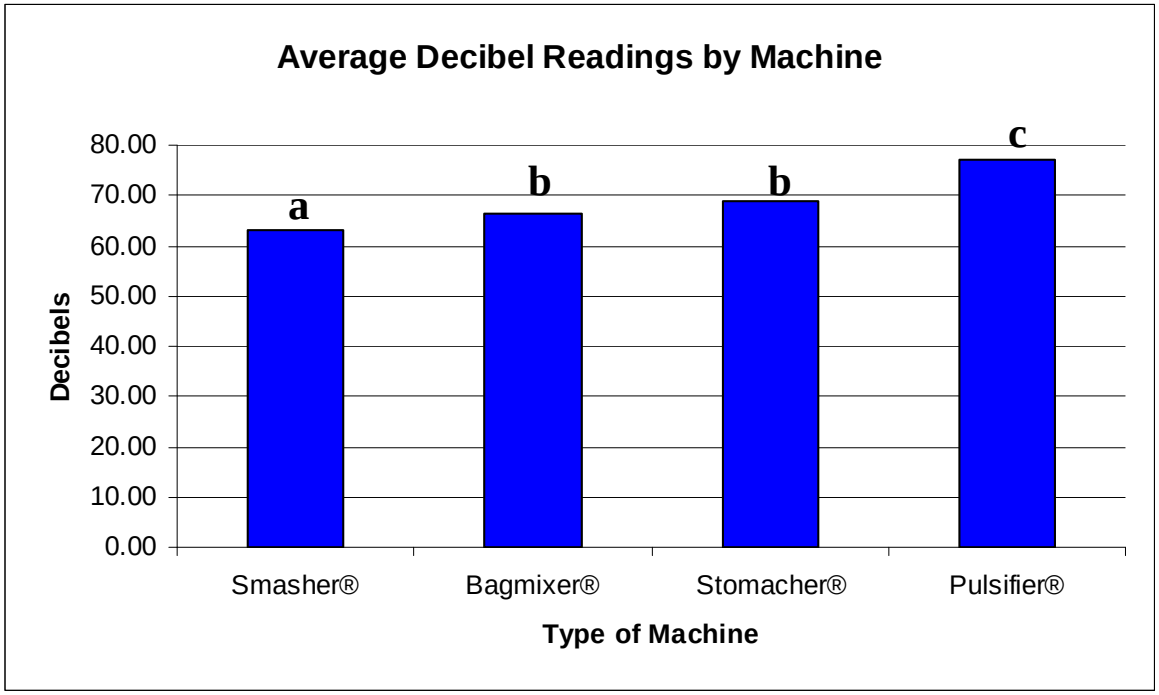
Table 5 Decibel emissions measured at a distance of five feet (1.52 m) from each instrument while in operation.

Machine	Food	Decibels
Stomacher	G. Beef	66
Stomacher	H. Dogs	71
Stomacher	S.Leaves	70
Stomacher	A. Sprouts	65
Stomacher	C. Sticks	66
Stomacher	C. Wings	68
Stomacher	F. Meat	68
Stomacher	Tofu	70
Stomacher	Peanuts	74
Smasher	G. Beef	62
Smasher	H. Dogs	63
Smasher	S.Leaves	63
Smasher	A. Sprouts	63
Smasher	C. Sticks	63
Smasher	C. Wings	63
Smasher	F. Meat	62
Smasher	Tofu	63
Smasher	Peanuts	66
Bagmixer	G. Beef	65
Bagmixer	H. Dogs	66
Bagmixer	S.Leaves	65
Bagmixer	A. Sprouts	67
Bagmixer	C. Sticks	67
Bagmixer	C. Wings	66
Bagmixer	F. Meat	67
Bagmixer	Tofu	67

Bagmixer	Peanuts	68
Pulsifier	G. Beef	76
Pulsifier	H. Dogs	78
Pulsifier	S.Leaves	76
Pulsifier	A. Sprouts	72
Pulsifier	C. Sticks	77
Pulsifier	C. Wings	77
Pulsifier	F. Meat	78
Pulsifier	Tofu	86
Pulsifier	Peanuts	74

(Table 5, continued)

Figure 3 Average of Decibel readings measured by Decibel Meter (Music Flash Sound Level Meter M. 33-1028) located at five feet (1.52 meters) of each instrument. The data corresponds to the average Decibel readings of each instrument for the food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Tofu, and Peanuts. N= 9 observations per machine



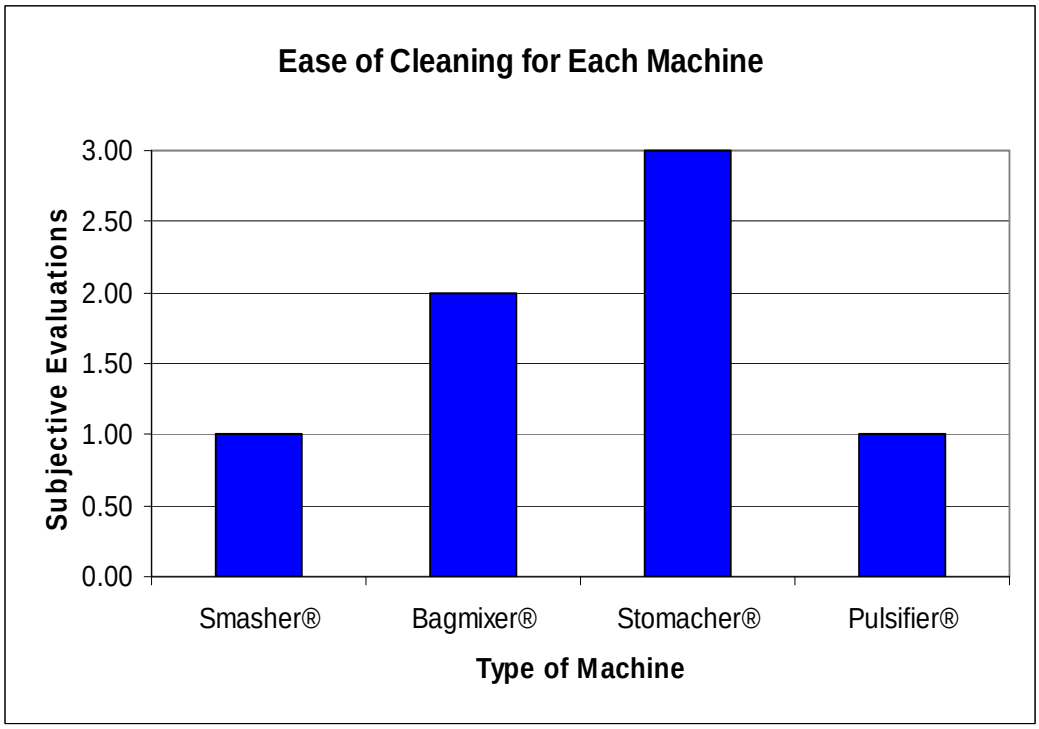
abcData series in Bars with same letter are not statistically different, whereas data series in bars with different letters are statistically different ($P<0.05$)

Standard error=0.84

Table 6 Ease of cleaning for each of the instruments evaluated.

Machine	Ease of Cleaning
Stomacher	3
Smasher	1
Bagmixer	2
Pulsifier	1

Figure 4 Average of Ease of Cleaning , measured by four laboratory personnel of each instrument. The data corresponds to the average subjective evaluations of each instrument for the food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Tofu, and Peanuts. N= 1 observation per machine



Conclusions

1. In regards to Viable Cell Counts, there is no significant difference amongst the four instruments evaluated. The Aerobic Plate Counts Petrifilms™ were consistently higher than the *E. coli* Coliform Counts Petrifilms™.

2. In regards to Noise Level measured by the Decibel Meter, there is a significant difference amongst the instruments. The instruments can be arranged as follows from quietest to loudest:
   1. Smasher®
   2. Bagmixer®, and Stomacher®
   3. Pulsifier®

   Note: the difference in Decibel emissions between the Bagmixer®, and the Stomacher® are not statistically significant (P=0.07).

3. In reference to the acceptable Noise Exposure standards determined by the United States Occupational Safety and Health Administration (OSHA), none of the instruments evaluated constitute an auditory health hazard.

4. In regards to ease of cleaning, the instruments can be ranked as follows, from easiest to most difficult to clean:
   1. Smasher®, and Pulsifier®
   2. Bagmixer®
   3. Stomacher®

   Note: the Smasher® and the Pulsifier® are equally easier to clean.

CHAPTER 3 - Materials and Methods

The Blender Smasher® and Interscience Bagmixer® were loaned from AES-Chemunex. The Pulsifier® was loaned by Microbiology International and the Stomacher® was a standard equipment at KSU Food Microbiology Laboratory Call Hall 202. A picture of all four instruments, from left to right, in the following order: Smasher®, Pulsifier®, Bagmixer®, and Stomacher® was taken (Figure 5). In total, three data sets for the parameters evaluated for each machine were obtained.

The performance of the four instruments was evaluated in regards to the following parameters: Viable Cell Counts, Noise Levels, Ease of Cleaning, and Ergonomics. The viable cell count (VCC) was ascertained by the use of Aerobic Plate Count (APC), and *E coli* Coliforms Count (ECC) Petrifilms™ (3M, St. Paul, MN). The scales used to evaluate the Noise levels,Eease of Cleaning and Ergonomics were based upon the Stomacher® as a reference point. Additionally, the same abbreviations than in the uninoculated study were used to denote the food samples utilized with the addition of Milk.

The four instruments evaluated in the project, namely the Stomacher®, Pulsifier®, Bagmixer®, and the Stomacher® were subjected to the same procedures on the trials of the experiment.

Figure 5 Instruments used in the experiment. From left to right, the Smasher® (AES-Chemunex, 1 Corporate Drive, Cranbury NJ), Pulsifier® (Microbiology International, 5111 Pegasus Court, Suite H, Frederick MD), Bagmixer® (Interscience, 301 Winter ST, Hanover MA), and Stomacher® (Seward Laboratory Systems Inc, 1648 Locust Avenue, Bohemia NY).

A. Viable Cell Counts

1. A sample of 25 g of food was placed with 225 mL of diluent (224.5 mL of sterile 0.1 % Bacto™ Peptone (Becton, Dickinson, and Co., Sparks, MD) water + 0.5 mL of *E. coli* inoculum at approximately 8.7 logs), of each: G. Beef, hot dog, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts.

2. The food samples were acquired at the local Walmart and Dillons grocery stores. The choice of food is related to the consistency of food as well as with bones to stimulate different types of food to be tested in these instruments. The inoculum culture was grown overnight into Bacto™ Tryptic Soy Broth (Becton, Dickinson, and Co., Sparks, MD) from the stock of lyophilized pellets available at the Food Microbiology Laboratory at Kansas State University. The lyophilized pellets used were the Easy Pellet® brand from Microbiologics (Saint Cloud, Mn).

3. All samples were treated for 60 seconds at medium speeds of each instrument for each sample.

4. Standard plate counts, as well as Coliform of each sample were monitored in duplicates by using Aerobic Plate Count (APC) and E coli Coliform Count (ECC) Petrifilm™ media.

In order to obtain a countable range of Colony Forming Units (CFU), a predetermined dilution schedule was used (Table 7).

Table 7 Dilution Schedule for the Ten Food Samples. Food samples were acquired at local Walmart, and Dillons grocery stores. The microbiological analysis was performed by using APC , and ECC Petrifilms™ (3M, St. Paul, MN).

Type of Food	Dilutions to be Plated	Type of Petrifilm™ media (1mL plated)
G. Beef	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
Hot Dog	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
S. Leaves	10^{-5},10^{-6},10^{-7}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
A. Sprouts	10^{-5},10^{-6},10^{-7}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
C. Sticks	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
C. Wings	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
F. Meat	10^{-3},10^{-4},10^{-5}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
Milk	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
Tofu	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)
Peanuts	10^{-4},10^{-5},10^{-6}	Aerobic Plate Count (APC)
	10^{-5},10^{-6},10^{-7}	*E coli* Coliform Count (ECC)

B. Noise Level measurements

Subjective measurements of Noise level each instrument were evaluated by four laboratory personnel standing at five feet (1.52 meters) in front and side perspectives and be ranked as:

1. Very Quiet
2. Quiet
3. Nearly Quiet
4. Acceptable Noise
5. Loud

Objective evaluations of Noise levels were performed by a decibel meter placed at five feet (1.52 meters) away to determine measurements on an ordinal scale. The decibel meter (Music Flash Sound Level Meter, Realistic®, M. 33-1028) was loaned from the KSU Physics department.

C. Ease of Cleaning

Ability to clean level of each instrument was evaluated by four laboratory personnel upon homogenization of all food samples. The ranking used to evaluate Ease of Cleaning was:

1. Very easy, complete and quick cleaning
2. Acceptable cleaning
3. Difficult

D. Ergonomics

Ergonomics of each instrument were evaluated by four laboratory personnel and ranked as:

1. Very user-friendly and ergonomic
2. Acceptable
3. Low ergonomics

E. Statistical Analysis

The statistical analysis of the data sets for the Viable Cell Counts, Noise Levels, Ease of Cleaning, and Ergonomics was performed with the Statistical Analytical Software (The SAS Institute, Cary, NC).

CHAPTER 4 - Results and Discussion

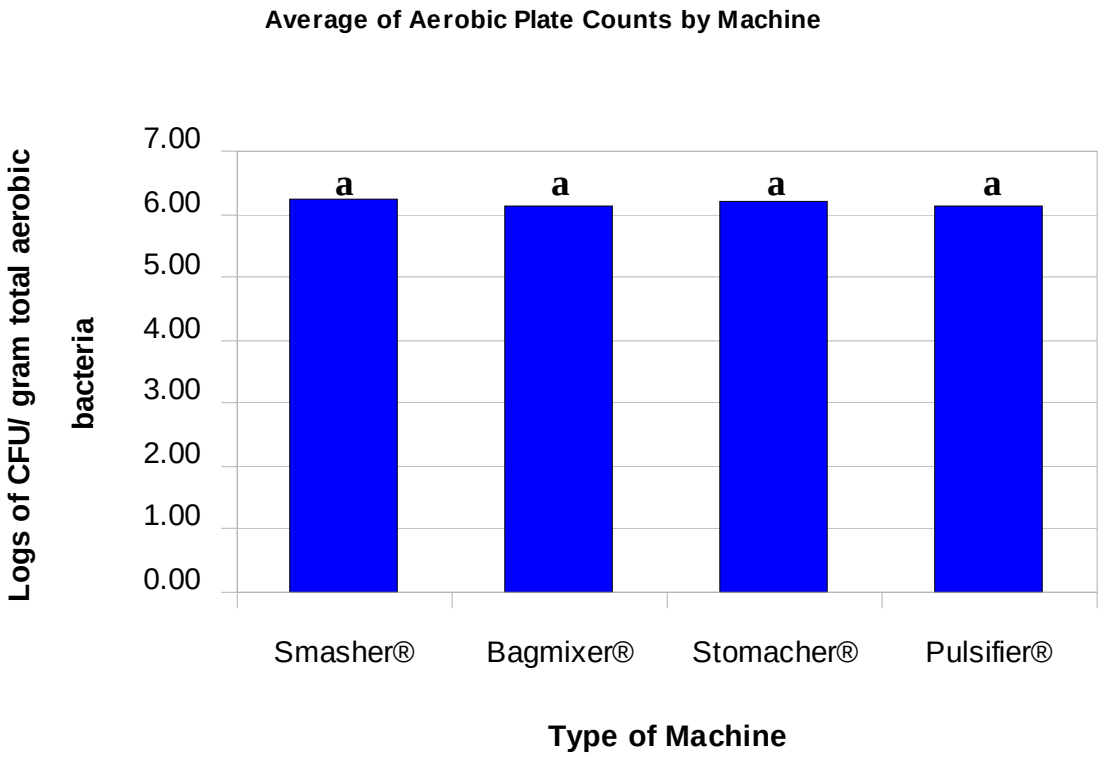
The characteristics to be evaluated by the study were: Viable Cell Counts, Noise levels, Ergonomics and Ease of Cleaning. In addition, a statistical analysis of interactions was carried out to determine whether the type of food had any significant influence on the viable cell counts and the decibel emissions.

The statistical analysis performed on the Viable Cell Counts obtained from the Aerobic Plate Counts (APC) and the *E. Coli* Coliform (ECC) counts demonstrated clear tendencies. Statistical analysis was done by applying the GLM procedure of the SAS (Cary, NC) program. Another command, procedure MIXED was executed as well. However, the results were the same using the GLM, or the MIXED procedure. The use of the GLM procedure was used in all of the statistical analysis, since it provides a more condensed set of data than procedure MIXED.

The average of the three data sets for the Viable Cell Counts in APC and ECC media denotes no statistical differences for the instruments evaluated (Figure 6 and, Figure 7). The data was obtained from APC, and ECC Petrifilms™ that were plated in duplicates (Table 8 and, Table 9). The hypothesis test yielded a average Logarithmic count of 6.06. The p-value for the variable corresponding to the type of instrument used was 0.46. A high p-value as the one obtained suggests that there is no significant difference in the performance of the instruments in regards to the Viable Cell Counts obtained.

Furthermore, the hypothesis test yields a p-value of <0.0001 for the media variable. The low p-value of the media variable suggests that the average Logarithmic count is not the same respective of the type of media employed. This difference can be attributed to the types of media used, specifically APC , and ECC. The mean logarithmic count for the APC media was 6.16, whereas the average Logarithmic count for the ECC media was 5.96. There was no significant interaction between the types of instruments, and the types of media used ($p=0.80$).

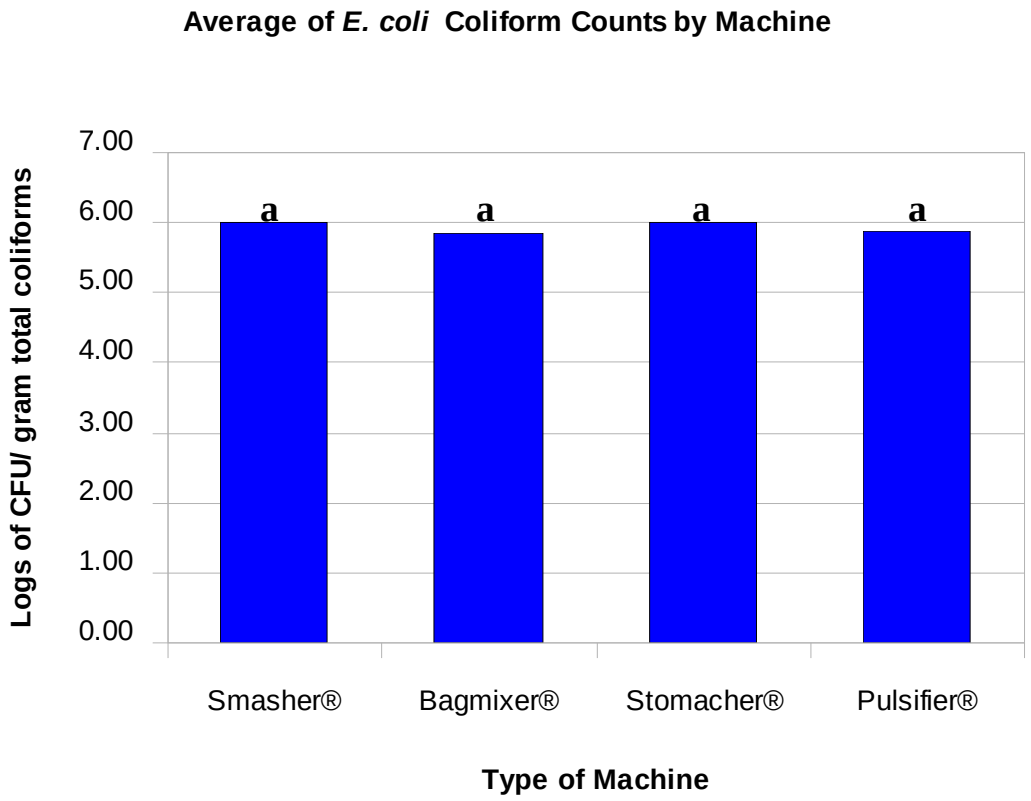
Figure 6 Average of 3 Aerobic Plate Counts datasets, measured as Logs of Aerobic CFU/g of sample of each instrument for: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. N=30 observations per machine



[a]Data series in Bars with same letter are not statistically different (P>0.05)

Standard error=0.03

Figure 7 Average of 3 *E. coli* Coliform Counts datasets, measured as Logs of Coliform CFU/g of food sample of each instrument for: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. N= 30 observations per machine



[a]Data series in Bars with same letter are not statistically different (P>0.05)

Standard error=0.03

Table 8 Viable cell counts in APC Petrifilm™ (3M, St. Paul, MN) corresponding to the three data sets used for the statistical analysis

Machine	Food	Inoc. Study #	Media	Logs
Stomacher	G. Beef	1	APC	6.39
Stomacher	H. Dogs	1	APC	5.93
Stomacher	S.Leaves	1	APC	5.74
Stomacher	A. Sprouts	1	APC	6.54
Stomacher	C. Sticks	1	APC	5.99
Stomacher	C. Wings	1	APC	6.70
Stomacher	F. Meat	1	APC	6.07
Stomacher	Milk	1	APC	5.92
Stomacher	Tofu	1	APC	5.71
Stomacher	Peanuts	1	APC	6.13
Stomacher	G. Beef	2	APC	5.98
Stomacher	H. Dogs	2	APC	6.26
Stomacher	S.Leaves	2	APC	5.97
Stomacher	A. Sprouts	2	APC	7.09
Stomacher	C. Sticks	2	APC	5.81
Stomacher	C. Wings	2	APC	6.16
Stomacher	F. Meat	2	APC	6.18
Stomacher	Milk	2	APC	5.86
Stomacher	Tofu	2	APC	6.14
Stomacher	Peanuts	2	APC	6.22
Stomacher	G. Beef	3	APC	6.06
Stomacher	H. Dogs	3	APC	6.08
Stomacher	S.Leaves	3	APC	5.97
Stomacher	A. Sprouts	3	APC	7.39
Stomacher	C. Sticks	3	APC	5.60
Stomacher	C. Wings	3	APC	6.38
Stomacher	F. Meat	3	APC	5.96

Stomacher	Milk	3	APC	6.33
Stomacher	Tofu	3	APC	6.38
Stomacher	Peanuts	3	APC	6.04
Smasher	G. Beef	1	APC	6.18
Smasher	H. Dogs	1	APC	6.39
Smasher	S.Leaves	1	APC	TNTC
Smasher	A. Sprouts	1	APC	6.64
Smasher	C. Sticks	1	APC	5.81
Smasher	C. Wings	1	APC	5.92
Smasher	F. Meat	1	APC	5.87
Smasher	Milk	1	APC	5.99
Smasher	Tofu	1	APC	6.86
Smasher	Peanuts	1	APC	6.01
Smasher	G. Beef	2	APC	5.93
Smasher	H. Dogs	2	APC	6.18
Smasher	S.Leaves	2	APC	6.19
Smasher	A. Sprouts	2	APC	6.98
Smasher	C. Sticks	2	APC	6.05
Smasher	C. Wings	2	APC	5.94
Smasher	F. Meat	2	APC	6.30
Smasher	Milk	2	APC	6.07
Smasher	Tofu	2	APC	6.22
Smasher	Peanuts	2	APC	6.26
Smasher	G. Beef	3	APC	6.18
Smasher	H. Dogs	3	APC	6.16
Smasher	S.Leaves	3	APC	6.30
Smasher	A. Sprouts	3	APC	7.17
Smasher	C. Sticks	3	APC	6.56
Smasher	C. Wings	3	APC	5.97
Smasher	F. Meat	3	APC	6.03

Smasher	Milk	3	APC	6.24
Smasher	Tofu	3	APC	6.06
Smasher	Peanuts	3	APC	6.16
Pulsifier	G. Beef	1	APC	6.48
Pulsifier	H. Dogs	1	APC	6.61
Pulsifier	S.Leaves	1	APC	5.59
Pulsifier	A. Sprouts	1	APC	6.47
Pulsifier	C. Sticks	1	APC	6.77
Pulsifier	C. Wings	1	APC	6.61
Pulsifier	F. Meat	1	APC	6.05
Pulsifier	Milk	1	APC	6.03
Pulsifier	Tofu	1	APC	5.93
Pulsifier	Peanuts	1	APC	6.05
Pulsifier	G. Beef	2	APC	6.03
Pulsifier	H. Dogs	2	APC	6.26
Pulsifier	S.Leaves	2	APC	6.09
Pulsifier	A. Sprouts	2	APC	7.24
Pulsifier	C. Sticks	2	APC	6.19
Pulsifier	C. Wings	2	APC	6.26
Pulsifier	F. Meat	2	APC	6.23
Pulsifier	Milk	2	APC	6.09
Pulsifier	Tofu	2	APC	6.23
Pulsifier	Peanuts	2	APC	6.13
Pulsifier	G. Beef	3	APC	5.76
Pulsifier	H. Dogs	3	APC	6.03
Pulsifier	S.Leaves	3	APC	6.07
Pulsifier	A. Sprouts	3	APC	6.41
Pulsifier	C. Sticks	3	APC	6.51
Pulsifier	C. Wings	3	APC	5.72
Pulsifier	F. Meat	3	APC	5.95

Pulsifier	Milk	3	APC	5.73
Pulsifier	Tofu	3	APC	5.62
Pulsifier	Peanuts	3	APC	5.90
Bagmixer	G. Beef	1	APC	6.26
Bagmixer	H. Dogs	1	APC	5.94
Bagmixer	S.Leaves	1	APC	TNTC
Bagmixer	A. Sprouts	1	APC	TNTC
Bagmixer	C. Sticks	1	APC	6.00
Bagmixer	C. Wings	1	APC	6.37
Bagmixer	F. Meat	1	APC	4.64
Bagmixer	Milk	1	APC	5.98
Bagmixer	Tofu	1	APC	6.33
Bagmixer	Peanuts	1	APC	6.08
Bagmixer	G. Beef	2	APC	6.24
Bagmixer	H. Dogs	2	APC	6.11
Bagmixer	S.Leaves	2	APC	5.94
Bagmixer	A. Sprouts	2	APC	7.06
Bagmixer	C. Sticks	2	APC	5.97
Bagmixer	C. Wings	2	APC	5.81
Bagmixer	F. Meat	2	APC	6.07
Bagmixer	Milk	2	APC	6.06
Bagmixer	Tofu	2	APC	6.30
Bagmixer	Peanuts	2	APC	5.97
Bagmixer	G. Beef	3	APC	6.03
Bagmixer	H. Dogs	3	APC	5.99
Bagmixer	S.Leaves	3	APC	5.86
Bagmixer	A. Sprouts	3	APC	6.75
Bagmixer	C. Sticks	3	APC	6.59
Bagmixer	C. Wings	3	APC	5.96
Bagmixer	F. Meat	3	APC	5.95

Bagmixer	Milk	3	APC	6.02
Bagmixer	Tofu	3	APC	5.78
Bagmixer	Peanuts	3	APC	5.86

(Table 8, continued)

Table 9 Viable cell counts in ECC Petrifilm™ (3M, St. Paul, MN) corresponding to the three data sets used in the statistical analysis.

Machine	Food	Inoc. Study #	Media	Logs
Stomacher	G. Beef	2	ECC	5.81
Stomacher	H. Dogs	2	ECC	6.17
Stomacher	S.Leaves	2	ECC	5.66
Stomacher	A. Sprouts	2	ECC	6.83
Stomacher	C. Sticks	2	ECC	5.58
Stomacher	C. Wings	2	ECC	5.98
Stomacher	F. Meat	2	ECC	6.27
Stomacher	Milk	2	ECC	5.53
Stomacher	Tofu	2	ECC	5.88
Stomacher	Peanuts	2	ECC	6.02
Stomacher	G. Beef	3	ECC	5.70
Stomacher	H. Dogs	3	ECC	6.16
Stomacher	S.Leaves	3	ECC	6.06
Stomacher	A. Sprouts	3	ECC	6.72
Stomacher	C. Sticks	3	ECC	5.56
Stomacher	C. Wings	3	ECC	5.68
Stomacher	F. Meat	3	ECC	5.95
Stomacher	Milk	3	ECC	6.21
Stomacher	Tofu	3	ECC	6.23
Stomacher	Peanuts	3	ECC	5.94
Smasher	G. Beef	2	ECC	5.66
Smasher	H. Dogs	2	ECC	5.81
Smasher	S.Leaves	2	ECC	5.78
Smasher	A. Sprouts	2	ECC	6.69
Smasher	C. Sticks	2	ECC	5.51
Smasher	C. Wings	2	ECC	5.72
Smasher	F. Meat	2	ECC	5.90

Smasher	Milk	2	ECC	5.58
Smasher	Tofu	2	ECC	5.86
Smasher	Peanuts	2	ECC	6.03
Smasher	G. Beef	3	ECC	6.20
Smasher	H. Dogs	3	ECC	6.03
Smasher	S.Leaves	3	ECC	6.19
Smasher	A. Sprouts	3	ECC	6.62
Smasher	C. Sticks	3	ECC	6.49
Smasher	C. Wings	3	ECC	5.93
Smasher	F. Meat	3	ECC	6.06
Smasher	Milk	3	ECC	6.02
Smasher	Tofu	3	ECC	6.03
Smasher	Peanuts	3	ECC	5.99
Pulsifier	G. Beef	2	ECC	5.53
Pulsifier	H. Dogs	2	ECC	5.87
Pulsifier	S.Leaves	2	ECC	5.83
Pulsifier	A. Sprouts	2	ECC	6.94
Pulsifier	C. Sticks	2	ECC	5.66
Pulsifier	C. Wings	2	ECC	5.64
Pulsifier	F. Meat	2	ECC	6.20
Pulsifier	Milk	2	ECC	5.72
Pulsifier	Tofu	2	ECC	6.03
Pulsifier	Peanuts	2	ECC	5.76
Pulsifier	G. Beef	3	ECC	5.58
Pulsifier	H. Dogs	3	ECC	5.81
Pulsifier	S.Leaves	3	ECC	5.88
Pulsifier	A. Sprouts	3	ECC	6.18
Pulsifier	C. Sticks	3	ECC	6.15
Pulsifier	C. Wings	3	ECC	5.66
Pulsifier	F. Meat	3	ECC	5.73

Pulsifier	Milk	3	ECC	5.90
Pulsifier	Tofu	3	ECC	5.56
Pulsifier	Peanuts	3	ECC	5.83
Bagmixer	G. Beef	2	ECC	5.66
Bagmixer	H. Dogs	2	ECC	6.01
Bagmixer	S.Leaves	2	ECC	5.72
Bagmixer	A. Sprouts	2	ECC	6.86
Bagmixer	C. Sticks	2	ECC	5.26
Bagmixer	C. Wings	2	ECC	5.20
Bagmixer	F. Meat	2	ECC	5.86
Bagmixer	Milk	2	ECC	5.68
Bagmixer	Tofu	2	ECC	5.48
Bagmixer	Peanuts	2	ECC	5.51
Bagmixer	G. Beef	3	ECC	5.91
Bagmixer	H. Dogs	3	ECC	5.91
Bagmixer	S.Leaves	3	ECC	5.91
Bagmixer	A. Sprouts	3	ECC	6.40
Bagmixer	C. Sticks	3	ECC	6.16
Bagmixer	C. Wings	3	ECC	6.04
Bagmixer	F. Meat	3	ECC	5.95
Bagmixer	Milk	3	ECC	6.03
Bagmixer	Tofu	3	ECC	5.60
Bagmixer	Peanuts	3	ECC	5.64
Stomacher	G. Beef	4	ECC	6.45
Stomacher	H. Dogs	4	ECC	6.29
Stomacher	S.Leaves	4	ECC	6.00
Stomacher	A. Sprouts	4	ECC	6.33
Stomacher	C. Sticks	4	ECC	5.59
Stomacher	C. Wings	4	ECC	5.95
Stomacher	F. Meat	4	ECC	5.76

Stomacher	Milk	4	ECC	5.95
Stomacher	Tofu	4	ECC	5.54
Stomacher	Peanuts	4	ECC	6.17
Smasher	G. Beef	4	ECC	7.25
Smasher	H. Dogs	4	ECC	6.11
Smasher	S.Leaves	4	ECC	5.77
Smasher	A. Sprouts	4	ECC	5.80
Smasher	C. Sticks	4	ECC	5.45
Smasher	C. Wings	4	ECC	6.40
Smasher	F. Meat	4	ECC	6.06
Smasher	Milk	4	ECC	5.77
Smasher	Tofu	4	ECC	5.00
Smasher	Peanuts	4	ECC	5.80
Pulsifier	G. Beef	4	ECC	6.43
Pulsifier	H. Dogs	4	ECC	6.04
Pulsifier	S.Leaves	4	ECC	6.02
Pulsifier	A. Sprouts	4	ECC	6.41
Pulsifier	C. Sticks	4	ECC	5.45
Pulsifier	C. Wings	4	ECC	6.36
Pulsifier	F. Meat	4	ECC	5.94
Pulsifier	Milk	4	ECC	5.45
Pulsifier	Tofu	4	ECC	TNTC
Pulsifier	Peanuts	4	ECC	6.22
Bagmixer	G. Beef	4	ECC	6.79
Bagmixer	H. Dogs	4	ECC	6.60
Bagmixer	S.Leaves	4	ECC	6.04
Bagmixer	A. Sprouts	4	ECC	6.19
Bagmixer	C. Sticks	4	ECC	5.81
Bagmixer	C. Wings	4	ECC	6.23
Bagmixer	F. Meat	4	ECC	6.00

Bagmixer	Milk	4	ECC	5.72
Bagmixer	Tofu	4	ECC	TNTC
Bagmixer	Peanuts	4	ECC	5.93

(Table 9, continued)

In inoculated experiment 1, the data set corresponding to the Viable Cell Counts on ECC petrifilms™ yielded Logarithmic counts expressed as Too Numerous To Count (TNTC). For this reason, this data set could not be used for the statistical analysis. The data set for the Aerobic Plate Counts measured by the APC petrifilms™ ,however , was in the countable range. Inoculated experiments 2, and 3 yielded 2 datasets for Aerobic Plate Count, and *E. coli* Coliform counts.

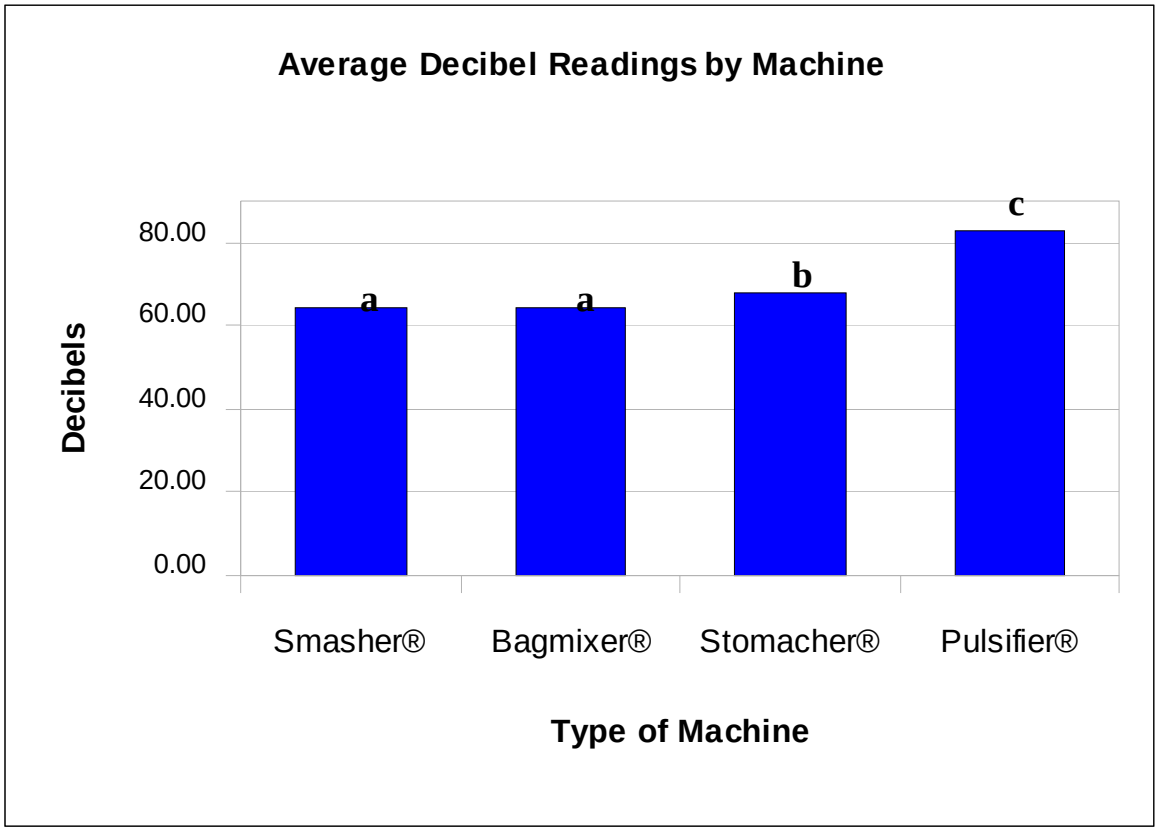
In order to have 3 datasets for Aerobic Plate Counts, and *E. coli* Coliform counts, inoculated experiment 4 was done to obtain *E. coli* Coliforms only, as measured by ECC petrifilms™.

A statistical test by using the GLM procedure in SAS (Cary, NC) was performed in order to inquire if inoculated experiments 1, and 4 constituted significant sources of variation to alter the value of the test statistic measured. The results of this procedure revealed that inoculated experiment 1, and 4 did not alter the value of the test statistic and did not constitute a significant source of variation respective to the trends of the results.

The data set that was used for the analysis of the decibels produced by each instrument during operation revealed consistent results as well (Figure 8). The data set that was used for the hypothesis test in regards to noise levels encompassed the data sets obtained in inoculated studies 1, 2, and 3 of the experiment (Table 10).

The hypothesis test by SAS revealed a mean decibel level of 69.99. The p-value for the instrument variable was <0.0001. These results are consistent with a strong evidence to support the claim that, on average, the noise level between the instruments is different. The average value for the decibel produced by each instrument while in operation was as follows: Smasher ® 64.60, Bagmixer® 64.50, Stomacher® 68.00, and Pulsifier® 82.86. Furthermore, the statistical analysis shows that there is no significant difference in the mean value of decibels produced by the Smasher® and the Bagmixer®.

Figure 8 Average of 3 Decibel readings datasets measured by Decibel Meter (Music Flash Sound Level Meter M. 33-1028) located at five feet (1.52 meters) of each instrument. The data corresponds to the average Decibel readings of each instrument for the ten food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. N= 30 observations per machine.



abcData series in the bars with same letters are not statistically different, while data series in bars with different letters are statistically different ($P<0.05$)

Standard error=0.48

Table 10 Decibel readings by Decibel Meter (Music Flash Sound Level Meter, Realistic®, M. 33-1028) located at five feet (1.52 meters) of each instrument.

Machine	Food	Inoc. Study #	Decibels
Stomacher	G. Beef	1	67
Stomacher	H. Dogs	1	67
Stomacher	S.Leaves	1	65
Stomacher	A. Sprouts	1	67
Stomacher	C. Sticks	1	68
Stomacher	C. Wings	1	66
Stomacher	F. Meat	1	69
Stomacher	Milk	1	67
Stomacher	Tofu	1	67
Stomacher	Peanuts	1	72
Stomacher	G. Beef	2	64
Stomacher	H. Dogs	2	65
Stomacher	S.Leaves	2	70
Stomacher	A. Sprouts	2	65
Stomacher	C. Sticks	2	69
Stomacher	C. Wings	2	65
Stomacher	F. Meat	2	68
Stomacher	Milk	2	67
Stomacher	Tofu	2	66
Stomacher	Peanuts	2	72
Stomacher	G. Beef	3	66
Stomacher	H. Dogs	3	68
Stomacher	S.Leaves	3	72
Stomacher	A. Sprouts	3	67
Stomacher	C. Sticks	3	74
Stomacher	C. Wings	3	66
Stomacher	F. Meat	3	72

Stomacher	Milk	3	66
Stomacher	Tofu	3	67
Stomacher	Peanuts	3	76
Smasher	G. Beef	1	64
Smasher	H. Dogs	1	64
Smasher	S.Leaves	1	63
Smasher	A. Sprouts	1	72
Smasher	C. Sticks	1	64
Smasher	C. Wings	1	64
Smasher	F. Meat	1	63
Smasher	Milk	1	63
Smasher	Tofu	1	65
Smasher	Peanuts	1	65
Smasher	G. Beef	2	62
Smasher	H. Dogs	2	64
Smasher	S.Leaves	2	65
Smasher	A. Sprouts	2	65
Smasher	C. Sticks	2	65
Smasher	C. Wings	2	65
Smasher	F. Meat	2	64
Smasher	Milk	2	64
Smasher	Tofu	2	65
Smasher	Peanuts	2	66
Smasher	G. Beef	3	64
Smasher	H. Dogs	3	63
Smasher	S.Leaves	3	64
Smasher	A. Sprouts	3	65
Smasher	C. Sticks	3	65
Smasher	C. Wings	3	64
Smasher	F. Meat	3	65

Smasher	Milk	3	64
Smasher	Tofu	3	64
Smasher	Peanuts	3	68
Bagmixer	G. Beef	1	63
Bagmixer	H. Dogs	1	66
Bagmixer	S.Leaves	1	64
Bagmixer	A. Sprouts	1	67
Bagmixer	C. Sticks	1	65
Bagmixer	C. Wings	1	64
Bagmixer	F. Meat	1	64
Bagmixer	Milk	1	65
Bagmixer	Tofu	1	65
Bagmixer	Peanuts	1	65
Bagmixer	G. Beef	2	64
Bagmixer	H. Dogs	2	63
Bagmixer	S.Leaves	2	64
Bagmixer	A. Sprouts	2	64
Bagmixer	C. Sticks	2	64
Bagmixer	C. Wings	2	63
Bagmixer	F. Meat	2	64
Bagmixer	Milk	2	63
Bagmixer	Tofu	2	63
Bagmixer	Peanuts	2	64
Bagmixer	G. Beef	3	65
Bagmixer	H. Dogs	3	65
Bagmixer	S.Leaves	3	65
Bagmixer	A. Sprouts	3	66
Bagmixer	C. Sticks	3	65
Bagmixer	C. Wings	3	66
Bagmixer	F. Meat	3	64

Bagmixer	Milk	3	65
Bagmixer	Tofu	3	64
Bagmixer	Peanuts	3	66
Pulsifier	G. Beef	1	86
Pulsifier	H. Dogs	1	84
Pulsifier	S.Leaves	1	84
Pulsifier	A. Sprouts	1	89
Pulsifier	C. Sticks	1	81
Pulsifier	C. Wings	1	84
Pulsifier	F. Meat	1	87
Pulsifier	Milk	1	80
Pulsifier	Tofu	1	80
Pulsifier	Peanuts	1	82
Pulsifier	G. Beef	2	81
Pulsifier	H. Dogs	2	83
Pulsifier	S.Leaves	2	83
Pulsifier	A. Sprouts	2	85
Pulsifier	C. Sticks	2	78
Pulsifier	C. Wings	2	85
Pulsifier	F. Meat	2	78
Pulsifier	Milk	2	80
Pulsifier	Tofu	2	80
Pulsifier	Peanuts	2	83
Pulsifier	G. Beef	3	78
Pulsifier	H. Dogs	3	88
Pulsifier	S.Leaves	3	88
Pulsifier	A. Sprouts	3	76
Pulsifier	C. Sticks	3	78
Pulsifier	C. Wings	3	88
Pulsifier	F. Meat	3	92

Pulsifier	Milk	3	85
Pulsifier	Tofu	3	78
Pulsifier	Peanuts	3	82

(Table 10, continued)

Furthermore, statistical analysis was done to determine if there were significant interactions amongst the variables studied in the Viable Cell Count, and decibel emissions datasets.

In regards to interaction of variables with respect to Viable Cell Counts by each instrument, the type of food, type of media, and the type of instrument were considered. No interaction is noted in between the type of food, and the type of instrument ($p=0.92$), and in between the type of media, and the type of instrument ($p=0.82$).

In other words, there is not enough evidence to claim that a type of food, or a type of media yielded significantly better or worse Viable Cell Counts in one instrument than in another one. Moreover, no statistical interaction is noted in between the instrument, food and media variables. It can be noted that no particular choice of food, plated on a given type of media, yielded significantly different Viable Cell Counts on different instruments.

The analysis of statistical interactions concerning decibel emissions in between the type of food, and type of instrument revealed significant differences ($p=0.001$). Different types of food yielded significantly higher or lower decibel emissions in any of the instruments. Such results can be attributed to the differences in hardness of the ten food matrixes employed. The analysis shows a statistical interaction of the instrument variable in regards to decibel emissions ($p<0.0001$). Based on this value it can be noted that different instruments produce different decibel emissions. This claim is consistent with the decibel emissions data analyzed in the preceding pages.

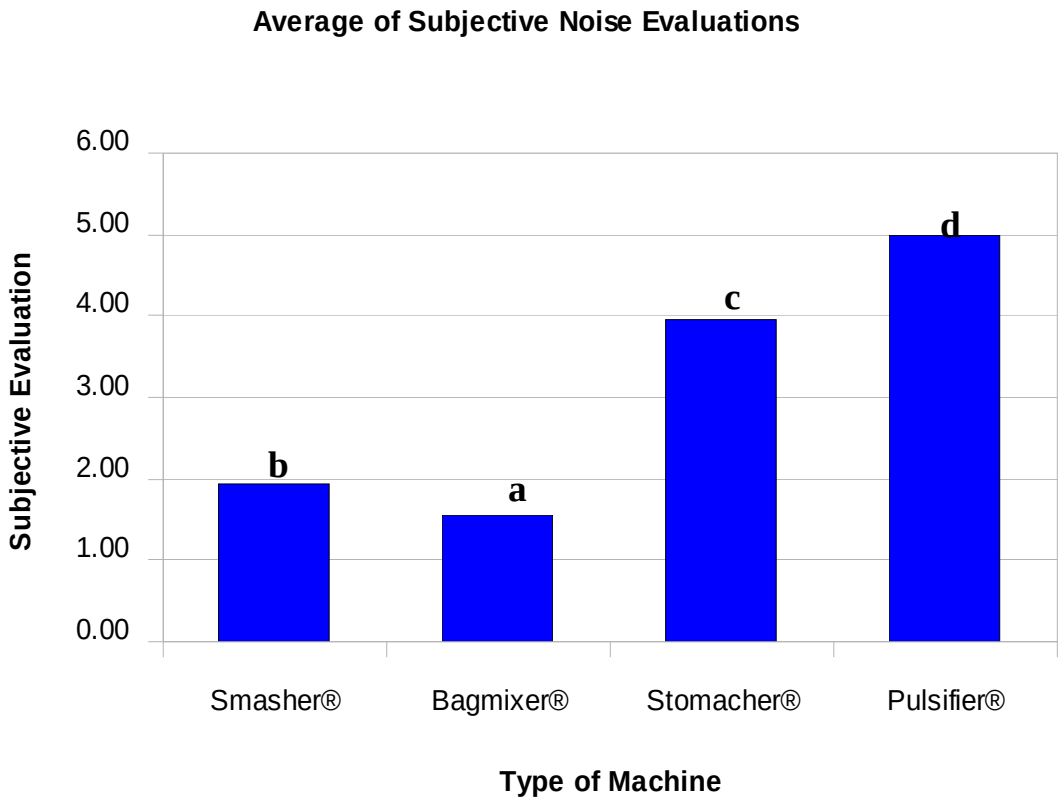
Ease of Cleaning, Ergonomics, and the Subjective Noise Evaluations measurements were also analyzed by the GLM procedure of SAS (Cary, NC). Noise levels were evaluated subjectively by means of a scale. A range from 1 to 5 was used. In the scale a value of 1 was used for very quiet, and 5 for loud.

The average of the sound evaluations by four laboratory personnel was obtained for each type of food for each instrument (Table 11). The average value for the subjective evaluations of noise exposure for each instrument was as follows: Bagmixer® 1.56, Smasher® 1.95, Stomacher® 3.97, and Pulsifier® 5.00 (Figure 9). With respect to the noise evaluations by laboratory personnel, the ranking of the instruments from quietest to loudest is as follows: Bagmixer®, Smasher®, Stomacher®, and Pulsifier®.

The average of the evaluations of Ease of Cleaning for each instrument was analyzed (Table 12).The analysis of Ease of Cleaning of each instrument was also assessed by a scale. The values of the scale ranged from 1 to 3. In the scale used, 1 was easy to clean, and 3 was difficult to clean. The average value for the Ease of Cleaning of each instrument was: Bagmixer® 1.83, Smasher® 1.33, Stomacher® 2.00, and Pulsifier® 2.33. According to the evaluations, the instruments can be ranked in the order of Ease of Cleaning as follows: Smasher®, Bagmixer®, Stomacher®, and Pulsifier® (Figure 10).

Lastly, Ergonomics values were obtained by averaging the evaluations of four laboratory personnel on the trials of the experiment (Table 13) .The analysis of the Ergonomics of each instrument was also quantified with the aid of a scale. The scale to analyze the Ergonomics of each instrument ranged from 1 to 3. In the scale, 1 stood for very user friendly and Ergonomic, and 3 stood for low Ergonomics. The average values for the instruments were as follows: Bagmixer® 1.00, as well as Smasher® 1.00, Stomacher® 2.33, and Pulsifier® 3.00. Based on the evaluations by the laboratory personnel, the ranking of the instruments in regards to Ergonomics is: Smasher® and Bagmixer®, followed by the Stomacher®, and then the Pulsifier® (Figure 11).

Figure 9 Average of 3 Subjective Evaluations of Noise Levels datasets, measured by four laboratory personnel located at five feet (1.52 meters) of each instrument. The data corresponds to the average subjective evaluations of each instrument for the ten food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. N= 3 observations per machine.



abcdData series in the bars with same letters are not statistically different, while data series in bars with different letters are statistically different ($P<0.05$)

Standard error= 0.05

Table 11 Data set used to evaluate the Noise levels as ascertained by laboratory personnel

Machine	Food	Inoc. Study #	Subj. Noise eval.
Stomacher	G. Beef	1	3.88
Stomacher	H. Dogs	1	4.13
Stomacher	S.Leaves	1	3.75
Stomacher	A. Sprouts	1	4.00
Stomacher	C. Sticks	1	4.25
Stomacher	C. Wings	1	4.00
Stomacher	F. Meat	1	4.25
Stomacher	Milk	1	4.63
Stomacher	Tofu	1	4.50
Stomacher	Peanuts	1	4.63
Stomacher	G. Beef	2	3.63
Stomacher	H. Dogs	2	3.63
Stomacher	S.Leaves	2	4.38
Stomacher	A. Sprouts	2	3.50
Stomacher	C. Sticks	2	4.00
Stomacher	C. Wings	2	3.75
Stomacher	F. Meat	2	3.88
Stomacher	Milk	2	3.50
Stomacher	Tofu	2	3.38
Stomacher	Peanuts	2	4.13
Stomacher	G. Beef	3	3.63
Stomacher	H. Dogs	3	3.63
Stomacher	S.Leaves	3	4.00
Stomacher	A. Sprouts	3	3.50
Stomacher	C. Sticks	3	4.00
Stomacher	C. Wings	3	3.50
Stomacher	F. Meat	3	4.25
Stomacher	Milk	3	3.63

Stomacher	Tofu	3	4.13
Stomacher	Peanuts	3	5.00
Smasher	G. Beef	1	2.00
Smasher	H. Dogs	1	2.13
Smasher	S.Leaves	1	1.75
Smasher	A. Sprouts	1	2.00
Smasher	C. Sticks	1	1.88
Smasher	C. Wings	1	1.75
Smasher	F. Meat	1	1.50
Smasher	Milk	1	1.25
Smasher	Tofu	1	2.00
Smasher	Peanuts	1	2.13
Smasher	G. Beef	2	1.75
Smasher	H. Dogs	2	1.88
Smasher	S.Leaves	2	1.75
Smasher	A. Sprouts	2	2.00
Smasher	C. Sticks	2	2.13
Smasher	C. Wings	2	2.00
Smasher	F. Meat	2	1.88
Smasher	Milk	2	1.75
Smasher	Tofu	2	1.75
Smasher	Peanuts	2	2.38
Smasher	G. Beef	3	1.75
Smasher	H. Dogs	3	2.00
Smasher	S.Leaves	3	2.13
Smasher	A. Sprouts	3	2.25
Smasher	C. Sticks	3	2.00
Smasher	C. Wings	3	1.88
Smasher	F. Meat	3	1.88
Smasher	Milk	3	2.13

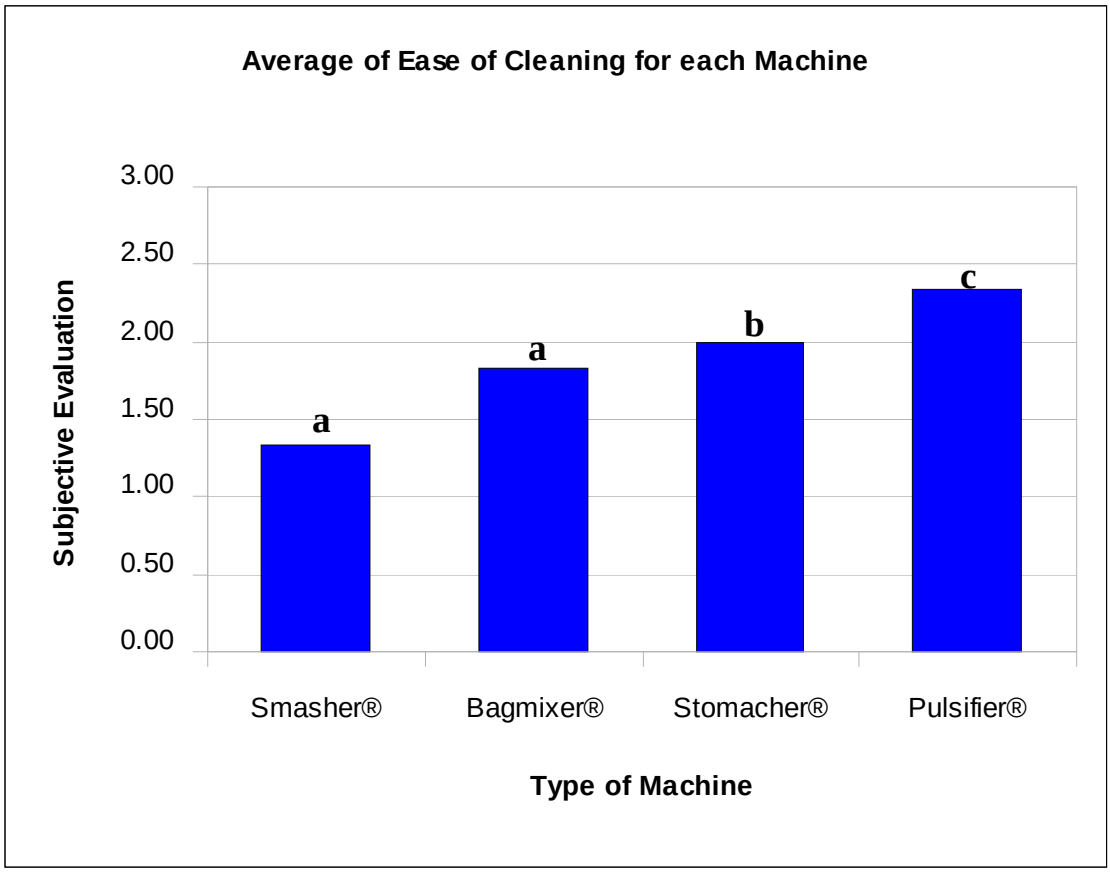
Smasher	Tofu	3	2.13
Smasher	Peanuts	3	2.75
Bagmixer	G. Beef	1	2.00
Bagmixer	H. Dogs	1	2.00
Bagmixer	S.Leaves	1	1.63
Bagmixer	A. Sprouts	1	2.25
Bagmixer	C. Sticks	1	1.88
Bagmixer	C. Wings	1	2.00
Bagmixer	F. Meat	1	1.75
Bagmixer	Milk	1	1.75
Bagmixer	Tofu	1	1.88
Bagmixer	Peanuts	1	1.88
Bagmixer	G. Beef	2	1.50
Bagmixer	H. Dogs	2	1.38
Bagmixer	S.Leaves	2	1.63
Bagmixer	A. Sprouts	2	1.00
Bagmixer	C. Sticks	2	1.00
Bagmixer	C. Wings	2	1.00
Bagmixer	F. Meat	2	1.00
Bagmixer	Milk	2	1.13
Bagmixer	Tofu	2	1.13
Bagmixer	Peanuts	2	1.63
Bagmixer	G. Beef	3	1.50
Bagmixer	H. Dogs	3	1.50
Bagmixer	S.Leaves	3	1.38
Bagmixer	A. Sprouts	3	1.50
Bagmixer	C. Sticks	3	1.50
Bagmixer	C. Wings	3	1.75
Bagmixer	F. Meat	3	1.75
Bagmixer	Milk	3	1.13

Bagmixer	Tofu	3	1.50
Bagmixer	Peanuts	3	1.75
Pulsifier	G. Beef	1	5.00
Pulsifier	H. Dogs	1	5.00
Pulsifier	S.Leaves	1	5.00
Pulsifier	A. Sprouts	1	5.00
Pulsifier	C. Sticks	1	5.00
Pulsifier	C. Wings	1	5.00
Pulsifier	F. Meat	1	5.00
Pulsifier	Milk	1	5.00
Pulsifier	Tofu	1	5.00
Pulsifier	Peanuts	1	5.00
Pulsifier	G. Beef	2	5.00
Pulsifier	H. Dogs	2	5.00
Pulsifier	S.Leaves	2	5.00
Pulsifier	A. Sprouts	2	5.00
Pulsifier	C. Sticks	2	5.00
Pulsifier	C. Wings	2	5.00
Pulsifier	F. Meat	2	5.00
Pulsifier	Milk	2	5.00
Pulsifier	Tofu	2	5.00
Pulsifier	Peanuts	2	5.00
Pulsifier	G. Beef	3	5.00
Pulsifier	H. Dogs	3	5.00
Pulsifier	S.Leaves	3	5.00
Pulsifier	A. Sprouts	3	5.00
Pulsifier	C. Sticks	3	5.00
Pulsifier	C. Wings	3	5.00
Pulsifier	F. Meat	3	5.00
Pulsifier	Milk	3	5.00

Pulsifier	Tofu	3	5.00
Pulsifier	Peanuts	3	5.00

(Table 11, continued).

Figure 10 Average of 3 Ease of Cleaning datasets, measured by four laboratory personnel of each instrument. The data corresponds to the average subjective evaluations of each instrument for the ten food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. N= 3 observations per machine.



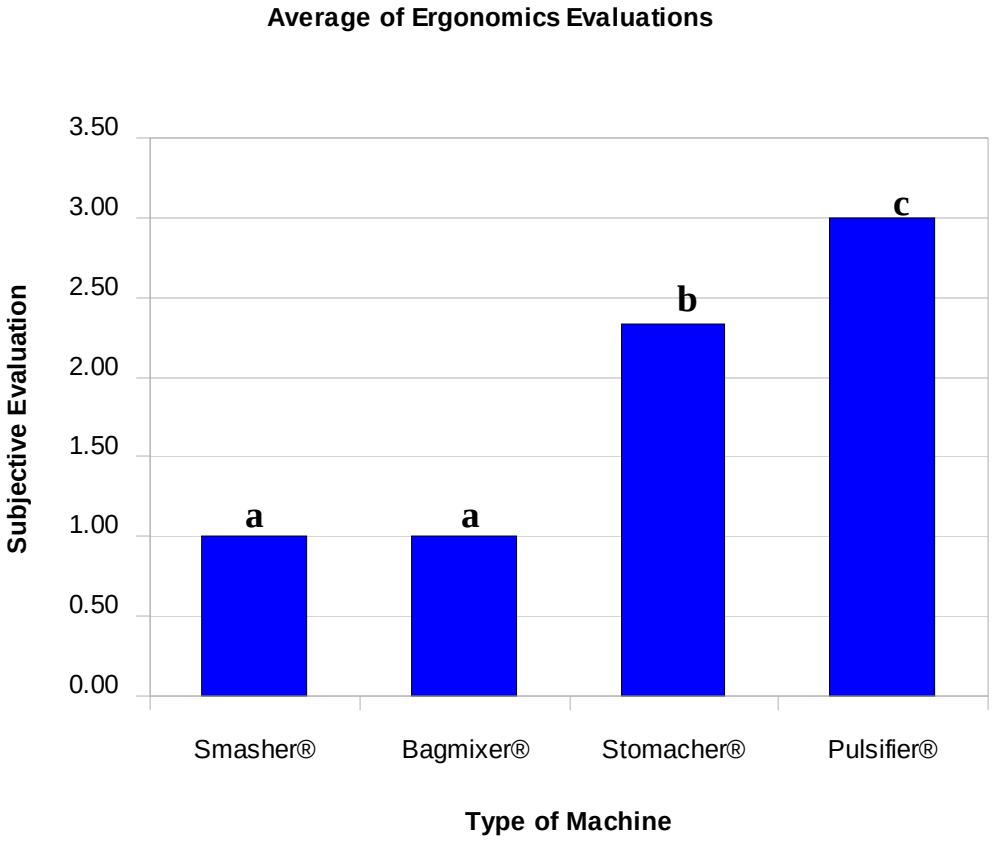
abcData series in the bars with same letters are not statistically different, while data series in bars with different letters are statistically different ($P<0.05$)

Standard error= 0.16

Table 12 Data set for the Ease of cleaning, as ascertained by four laboratory personnel.

Machine	Inoc. Study #	Cleaning
Stomacher	1	2
Stomacher	2	2
Stomacher	3	2
Smasher	1	2
Smasher	2	1
Smasher	3	1
Bagmixer	1	2
Bagmixer	2	1.5
Bagmixer	3	2
Pulsifier	1	3
Pulsifier	2	2
Pulsifier	4	2

Figure 11 Average of 3 Ergonomics ranking datasets, measured by four laboratory personnel of each instrument. The data corresponds to the average subjective evaluations of each instrument for the ten food samples: G. Beef, H. Dogs, S. Leaves, A. Sprouts, C. Sticks, C. Wings, F. Meat, Milk, Tofu, and Peanuts. Ergonomics was evaluated in regards to the extent of user friendliness of each instrument. N= 3 observations per machine.



abcData series in the bars with same letters are not statistically different, while data series in bars with different letters are statistically different ($P<0.05$)

Standard error= 0.14

Table 13 Data set for the evaluation of ergonomics, as ascertained by four laboratory personnel.

Machine	Inoc. Study #	Ergonomics
Stomacher	1	3
Stomacher	2	2
Stomacher	3	2
Smasher	1	1
Smasher	2	1
Smasher	3	1
Bagmixer	1	1
Bagmixer	2	1
Bagmixer	3	1
Pulsifier	1	3
Pulsifier	2	3
Pulsifier	4	3

CHAPTER 5 - Conclusions:

Conclusions from inoculated studies:

1. The Viable Cell Counts do not differ significantly amongst the four instruments. The types of media used were Aerobic Plate Counts (APC), and *E. coli*/Coliform Count (ECC) Petrifilms™. The APC counts were consistently a little higher than the ECC counts.

2. The analysis of the noise level measured in Decibels, and by laboratory personnel reveals there is a significant difference between the instruments. The subjective, and objective evaluations rank the Smasher®, and the Bagmixer® as the quietest instruments.

3. In regards to Ease of Cleaning, the only instrument that was moderately hard to clean was the Pulsifier®. However, it must be noticed that the Pulsifier® is better fitted for susbsequent PCR, and electrophoresis studies than the rest of the instruments evaluated since the mechanical pounding of the other instruments might lead to disruption of the matrix of the samples and release of compounds that can interfere with the PCR reaction

4. In regards to most Ergonomical, the Smasher®, and Bagmixer® instruments are equally better fitted than the rest

5. The four instruments are equally fit for recovery of Microorganisms in the types of media employed. Moreover, the statistical analysis denotes that no particular instrument was better suited for the types of food used in regards to Viable Cell Counts.

Overall Conclusions

1. The Viable Cell Counts do not differ significantly amongst the four instruments. The types of media used were Aerobic Plate Counts (APC), and *E. coli*/Coliform Count (ECC) Petrifilms™. The APC counts were consistently higher than the ECC counts.

2. The analysis of the noise level measured in Decibels, reveals that different instruments have different performance. In the uninoculated, as well as the inoculated studies the Smasher® ranks the quietest.

3. None of the instruments evaluated produce Decibel levels above the permissible limit determined by the United States Occupational Safety and Health Administration (OSHA).

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